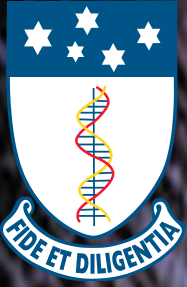

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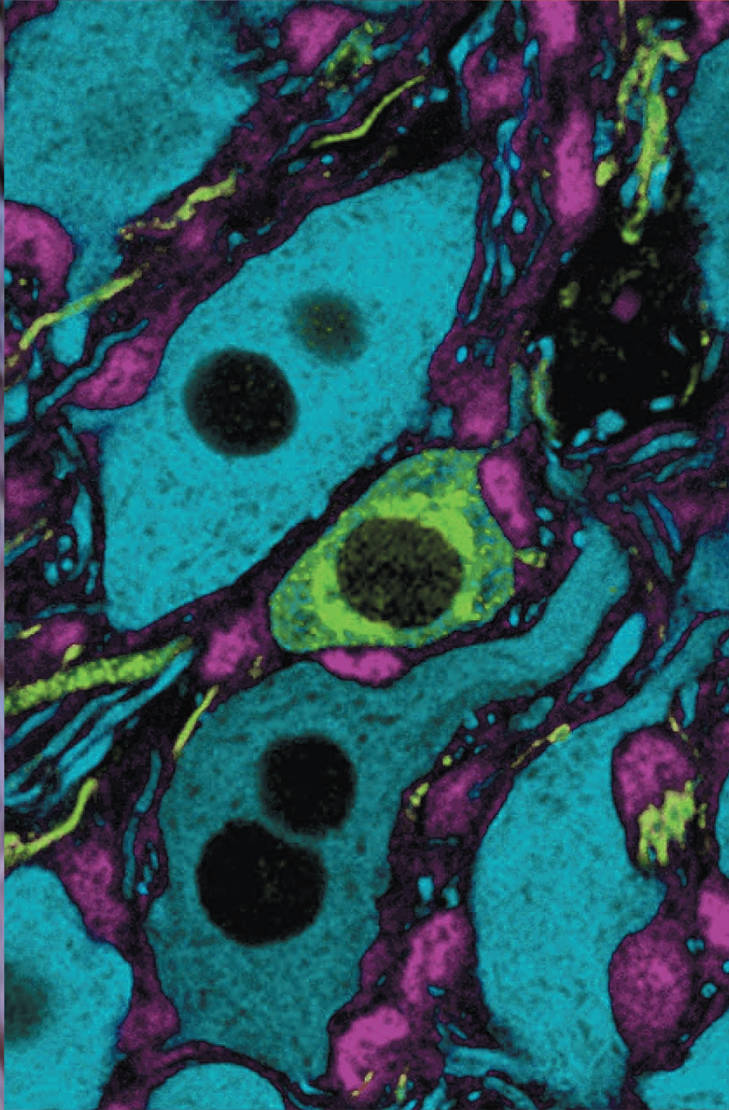

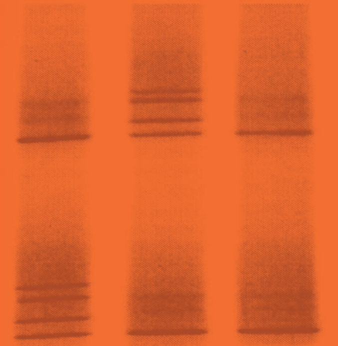

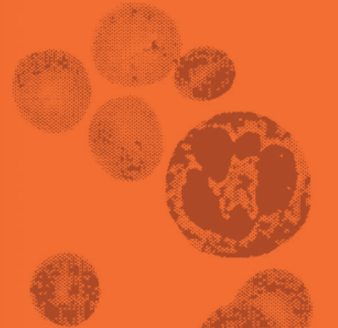

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REGULAR FEATURES

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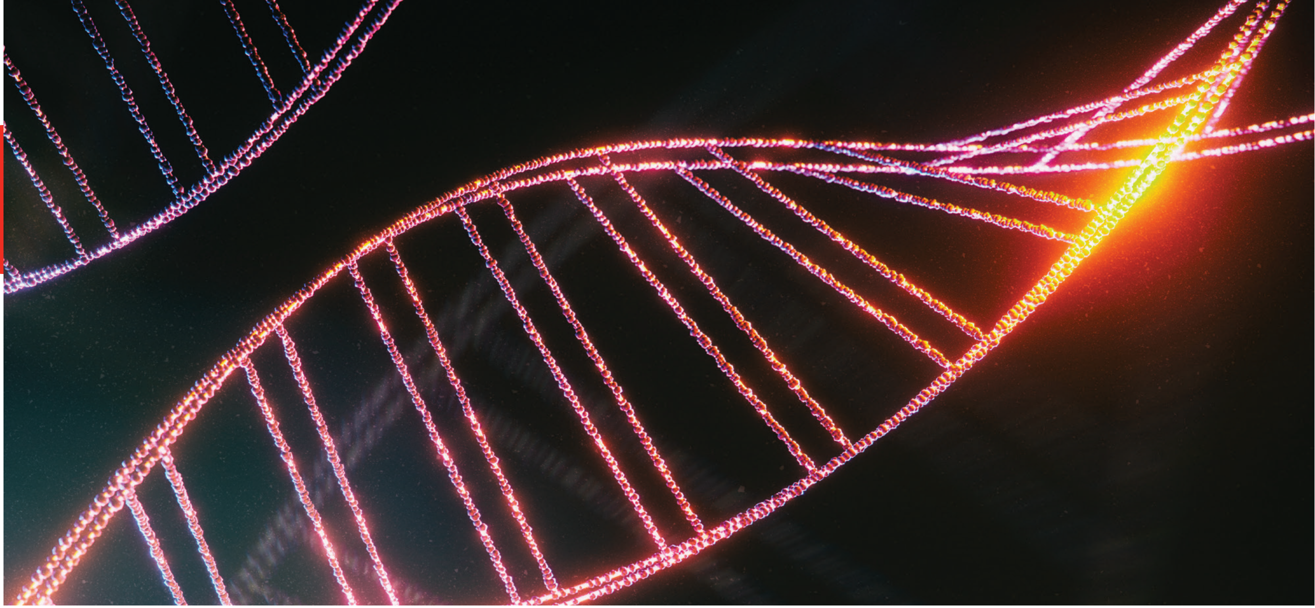

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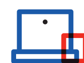

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ORIGINAL ARTICLE

β-lactam resistance in *Pseudomonas aeruginosa* and its detection in the microbiology laboratory

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Fellowship Dissertation
Microbiology Department, The Alfred Hospital, Melbourne, Victoria

Abstract

Pseudomonas aeruginosa is an environmental organism that is an important cause of serious and often life-threatening infection. Its array of virulence factors and resistance mechanisms can make it difficult to find a suitable antibiotic to treat infections. β-lactam antibiotics are commonly the first line treatment option for *P. aeruginosa* infections. This review will look at the mechanisms of β-lactamase resistance as well as the methods used to detect resistance in the microbiology laboratory. The microbiology laboratory has a key role in providing susceptibility information to allow treatment of infections, as well as providing essential data for infection prevention and surveillance strategies.

Key words: Pseudomonas aeruginosa, *β-lactam, drug resistance, bacterial, microbiology laboratory.*

Introduction

Pseudomonas aeruginosa is a Gram-negative rod that is regarded as an environmental organism. It is found notably in soil and water exposed to intense human activity (Crone *et al* 2020).

It is an opportunistic pathogen, capable of causing life-threatening infections in humans including pneumonia and infections in the skin and soft tissue, urinary tract and blood stream. It is commonly associated with healthcare acquired infections, infections in immunocompromised hosts, burns patients and chronic infections in patients with structural lung disease such as cystic fibrosis. Healthcare acquired infections with *P. aeruginosa* are an important cause of morbidity, mortality and increased health care costs.

P. aeruginosa is commonly isolated from water reservoirs in the hospital environment (Kizny Gordon *et al* 2017; Moloney *et al* 2020). Water reservoirs include sinks, drains, toilets, shower heads, flower vase water and cleaning and medical equipment. It has been shown to survive up to 8 weeks on floors and fabrics (Jabłońska-Trypuć *et al* 2022).

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Hospital drinking water has repeatedly been implicated as a source of healthcare acquired infection (Bicking Kinsey *et al* 2017; Cristina *et al* 2021; Hayward *et al* 2022).

Although *P. aeruginosa* is not considered part of the normal human microbiome, the intestinal tract is an important reservoir. While *P. aeruginosa* can be found in the intestinal flora of less than 5% of people in a non-clinical environment (Ruiz-Roldán *et al* 2018; Hu *et al* 2023) this figure can rise to 51% in residents of long term care facilities (Martak *et al* 2022) indicating a greatly increased likelihood of being colonised with *P. aeruginosa* in the healthcare setting.

Multiple risk factors for infection with resistant *P. aeruginosa* are commonly present, with each increasing the likelihood of infection with a resistant *P. aeruginosa* compared with a susceptible strain. Colonisation is a risk factor for subsequent infection, as is a known history of a previous *P. aeruginosa* infection. Residence in long-term care facilities or hospitalisations for extended periods of time predisposes patients to colonisation. This is especially true of facilities with high rates of resistant *P. aeruginosa*. In a large database study that evaluated risk factors for development of resistant *P. aeruginosa* infection, patients from hospitals with a resistant *P. aeruginosa* prevalence found that ≥ 4% were at greatest risk (Lodise *et al* 2019). Critical illness requiring prolonged intensive care and prolonged mechanical ventilation appear to be

particularly important healthcare associated risk factors for colonisation and infection. Previous exposure to anti-pseudomonal antibiotics, particularly carbapenems and fluoroquinolones, also contribute to increased risk for infection with a resistant *P. aeruginosa*. In addition to specific agent exposures, data suggest that cumulative antibiotic exposure increases the risk of infection with a resistant *P. aeruginosa* (Raman *et al* 2018).

The pathogenicity of *P. aeruginosa* can be attributed to a myriad of virulence factors, not the least of which is its metabolic versatility. It is capable of producing secondary metabolites and polymers using different carbon sources. This allows it to adapt to a wide variety of environments and opportunistically infect humans. Other important virulence factors that interfere with the effectiveness of antibiotics include biofilm formation, extracellular products and metabolites, outer membrane proteins, secretion systems and efflux pumps.

P. aeruginosa can develop resistance to antibiotics either through mutational processes that alter the expression and/or function of chromosomally encoded mechanisms or through the acquisition of resistance genes on mobile genetic elements and both strategies for developing drug resistance can severely limit the therapeutic options for serious infections. *P. aeruginosa* is capable of harbouring a wide range of antimicrobial resistance mechanisms, often simultaneously, leading to strains that are resistant to multiple antibiotic classes.

Carbapenems and β-lactam/β-lactamase inhibitor combinations have become critical tools in treating multi-resistant *P. aeruginosa* infections, but increasing resistance threatens their efficacy. Carbapenems are remarkably effective β-lactams and have a broad spectrum of activity. They are less vulnerable to most β-lactamases, including AmpC and the extended spectrum β-lactamases, making them a reliable treatment for most infections. Additionally they have fewer side effects and are safer to use than most other last-line antimicrobials such as colistin. The emergence and rapid world-wide spread of carbapenem resistance in particular, is a major global public healthcare issue.

The heatmap below (Figure 1) shows global rates of resistance to meropenem. While rates of meropenem resistance in Australia, indeed Oceania, remain low, countries around the world where people may travel have much higher rates of resistance. Data is also not collected in many countries (grey areas on the map) so the situation with regards to resistance from these areas is unknown. This is an important consideration for laboratories that may service returned travellers and underlines the importance of an accurate and detailed medical history and screening for resistant pathogens.

An increasing incidence of highly epidemic multidrug-resistant clones has been observed in many parts of the world (Aguilar-Rodea *et al* 2022). A common approach

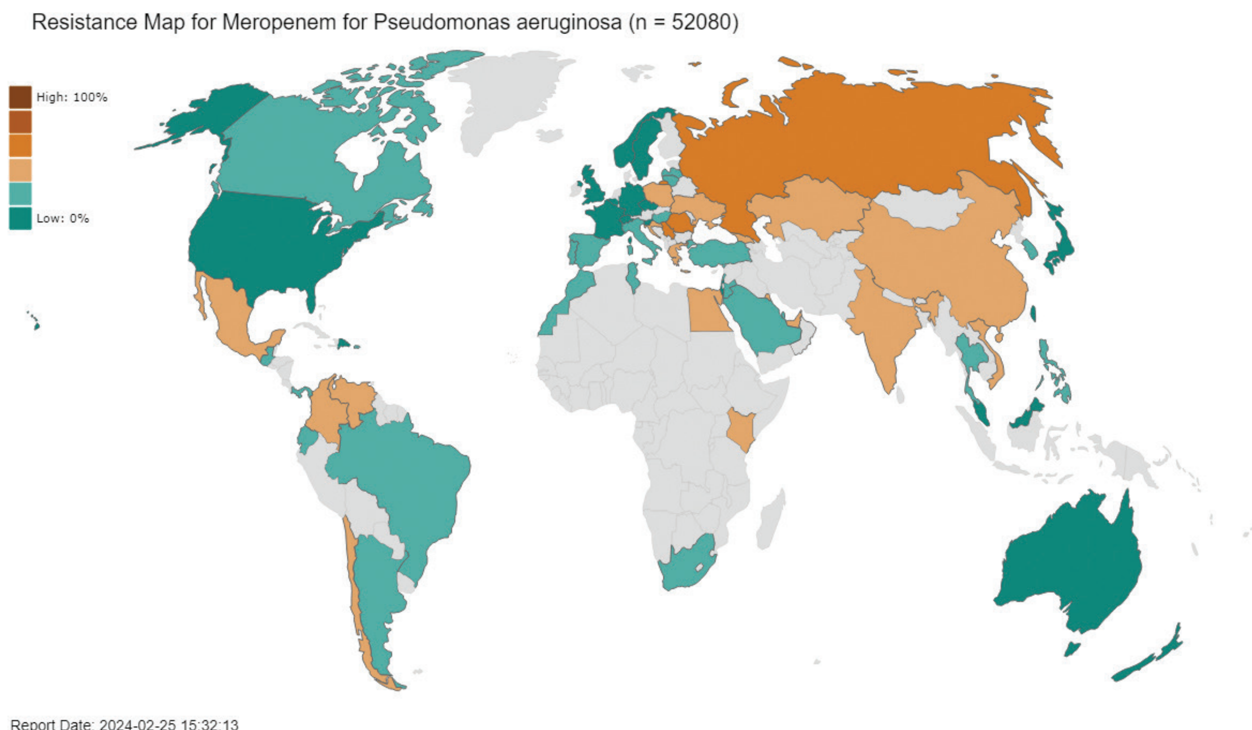

Figure 1. *Resistance heatmap for worldwide meropenem resistance 2022 using EUCAST guidelines (www.globalSMARTsite.com, accessed 8/4/2024)*

to studying *P. aeruginosa* populations is multilocus sequence typing (MLST) (Dufkova *et al* 2023). MLST is a form of genotyping that allows strain relatedness to be investigated. Each strain is given a number, preceded by the letters ST sequence type). While there have been more than 3000 STs described, there are a few that have been identified repeatedly in outbreak situations worldwide, for example ST235, ST111, and ST233 (Del Barrio-Tofiño *et al* 2020) with ST235 being the most widespread (Treepong *et al* 2018). These infections can become quickly life-threatening and have emerged as a global public health threat (Moradali *et al* 2017). Carbapenem resistant *P. aeruginosa* was placed in the Priority 1: critical category of pathogens for which research and development of new antibiotics is required in the World Health Organisation's Bacterial Priority Pathogens List (Tacconelli *et al* 2018). In 2019, the number of deaths attributable to and associated with resistance in *P. aeruginosa* was estimated at over 300,000 globally (Murray *et al* 2022) and the cost of hospital acquired resistant *P. aeruginosa* respiratory infection was higher than any other organism/infection combination (Lee *et al* 2021) and when resistant strains become widespread, the effectiveness of empirical therapy is reduced and the risk of poor outcome increases (Babich *et al* 2020). The mechanisms of resistance of *P. aeruginosa* are so diverse that covering them all is beyond the scope of this review. Specifically, this review will look at the resistance mechanisms associated with β-lactams and the detection of resistance in the moderately resourced microbiology laboratory.

Anti-pseudomonal β-lactam antibiotics

β-lactams are the mainstay of treatment for infections caused by *P. aeruginosa*. The agents that are currently available in Australia (as per Australian Register of Therapeutic Goods) with activity against *P. aeruginosa* are outlined in Table 1. The mechanism of action of β-lactam antibiotics is to interrupt bacterial cell-wall formation by bonding covalently to essential penicillin-binding proteins (PBPs). PBPs are involved in the synthesis of the layer of peptidoglycan that forms part of the cell wall and provides structural integrity to *P. aeruginosa* cells. PBPs are involved in the later stages of peptidoglycan synthesis and remodelling of peptidoglycan during cell growth and division. *P. aeruginosa* has eight PBPs, numbered 1a, 1b, 2, 3, 3a, 4, 5, and 7. Only PBP3 is essential for growth, which is main reason it is a target for the β-lactam antibiotics. The bactericidal properties of β-lactams are due to their ability to inhibit the activities of PBPs. Once the β-lactam has passed through the outer membrane porins and entered the active site of a PBP it covalently binds to the catalytic serine permanently, inactivating the PBP. The inhibition of PBPs can result in the reduction of the structural integrity of the cell wall resulting in cell lysis and death. The mode of administration can have a profound effect on the outcome of infection. Continuous infusion with meropenem was shown to enable better pharmacodynamic target achievement, leading to higher clinical improvement rate and lower mortality than usual intermittent dosing (Yu *et al* 2018). Using combination of agents has also been explored, especially against multidrug resistant and extensively drug resistant *P. aeruginosa* (Ribera *et al* 2015; Khawcharoenporn *et al* 2018; Montero *et al* 2018; Siriyong *et al* 2019) with reports of increased eradication of infection and improved survival of patients (Horcajada *et al* 2019).

Table 1. *Examples of anti-pseudomonal β-lactam antibiotics in use in Australia*

Class of β-lactam	Examples
Cephalosporins	ceftazidime, cefepime
Monobactams	aztreonam
Carbapenems	meropenem, imipenem (with cilastatin),
Combination β-lactam / β-lactamase inhibitors	piperacillin-tazobactam, ceftazidime-avibactam, ceftolozane-tazobactam

Combination β-lactam/β-lactamase inhibitors

β-lactamase inhibitors were designed to be administered with β-lactam antibiotics. They bind, often irreversibly, to the active site of β-lactamases, thereby preventing hydrolysis of the β-lactam. The newer inhibitors have a more stable interaction with certain enzymes than the older inhibitors, and their stability means fewer molecules are required to inhibit a β-lactamase.

Paucity of new anti-pseudomonal antibiotics

Despite resistant infections causing a significant number of deaths per year, development of new antibiotics has not kept pace with the emergence of resistance. The development of a new antimicrobials is time consuming and costly. Estimates put this at 13-20 years, requiring in excess of US$1 billion (Wouters *et al* 2020; Wouters *et al* 2022; World Health Organization 2022). The economic return on investment is prohibitively low compared with the development of other drugs, for example antineoplastic agents.

A report published in 2022 (World Health Organization 2022) reveals that 28 antibiotics with potential effect against *P. aeruginosa* are in the clinical development pipeline. When the likelihood of approval from Phase I is considered, only one in five of these antibiotics will make it to the approval stage (Hay *et al* 2014; Mullard 2016).

The "Netflix model" is one proposed solution to this problem. One 'subscription' program has already been rolled out in the UK, where the pharmaceutical company receives a fixed annual payment for the use of their product, which will be made irrespective of the amount of antibiotic that is actually used (Leonard *et al* 2023).

Definitions – MDR/XDR/PDR/DTR

Different terms such as multi-drug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) have been used to describe levels of drug resistance. A diversity of terminology appears in the literature, which can lead to confusion (Falagas *et al* 2006).

A joint initiative by the European Centre for Disease Prevention and Control and the Center for Disease Control and Prevention agreed to the following definitions shown in Table 2 (Magiorakos *et al* 2012).

More recently, the term difficult-to-treat (DTR) was introduced (Kadri *et al* 2018) and was assigned to organisms that were intermediate or resistant in vitro to all β-lactam categories (including carbapenems) and fluoroquinolones.

Mechanisms of resistance to β-lactams antibiotics

The wide resistance capacity of *P. aeruginosa* can be attributed to the following factors:

- the low permeability of the outer membrane (porins)
- the existence of several excretion systems (efflux pumps)
- the presence of enzymes (e.g. β-lactamases and carbapenemases)
- the synthesis of altered penicillin binding proteins with lower affinity to β-lactams.

Resistance to β-lactams in *P. aeruginosa* often involves a combination of these mechanisms (Pachori *et al* 2019).

Table 2. *Definitions used to describe* P. aeruginosa *resistance*

Multi-drug resistant	**MDR**	acquired non-susceptibility to at least one agent in three or more antimicrobial categories
Extensively drug resistant	**XDR**	non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories)
Pan drug resistant	**PDR**	non-susceptibility to all agents in all antimicrobial categories

Table 3. *Basic β-lactam resistance phenotypes*

Possible cause	Results in
Low outer membrane permeability combined with activation or derepression of efflux systems	Increase in minimum inhibitory concentration (MIC) for most β-lactams including meropenem but not imipenem
Derepression of AmpC	Resistance to all β-lactams except Carbapenems
OXA-type β-lactamases	Resistance to Penicillins and some Cephalosporins
Decreased OprD expression	Increase in MIC to Carbapenems, other β-lactams not affected
Production of plasmid or integron encoded extended spectrum beta lactamases (ESBLs) or carbapenemases from different molecular classes (eg KPC gene)	Resistance to all β-lactams
Presence of a VIM, IMP or NDM carbapenemase gene	Resistance to all β-lactams except aztreonam

Natural resistance

Natural resistance, also known as chromosomally encoded resistance, may be constitutive (always expressed in the species), or induced (the genes are naturally occurring in the bacteria but are only expressed at levels which create resistance after exposure to an antibiotic). Constitutive resistance is independent of previous antibiotic exposure and not related to horizontal gene transfer or mutations.

Porins

Exchange of nutrients across the outer membrane is orchestrated by proteins in a β-barrel structure producing water-filled diffusion channels known as porins. *P. aeruginosa* has very low numbers of porins on the outer membrane – 12 to100 fold lower than that of *Escherichia coli* (Yoshimura and Nikaido 1982), meaning it has very low permeability to antimicrobials based on its structure alone.

The main type of porin *P. aeruginosa* has on its outer membrane is OprF, the structure of which does not allow a wide range of antibiotics to enter the cell. *P. aeruginosa* is also able to alter the number and type of porin on its membrane, to a more specific porin (e.g. OprD) that allows entry of an even smaller range of compounds.

OprD is well-studied due to its involvement in the entry of carbapenems into the cell. It has been found in high numbers in biofilm outer membrane vesicles (OMVs) (Park *et al* 2015). OMVs are budding structures that contain periplasmic components and it is believed that by concentrating OprD in these structures, it leads to accumulation of carbapenems in the OMV and simultaneously lowers the drug concentration in the bacterial cell (Chevalier *et al* 2017). OprD expression is decreased in anaerobic environments, which is the case within biofilms that form in the lung of patients with chronic lung diseases (Jurado-Martín *et al* 2021). This may be a contributing factor to the enhanced resistance to carbapenems in biofilms (Tata *et al* 2016).

Efflux pumps

Efflux pumps protect bacteria from toxic compounds produced by other species of bacteria or by the host, antimicrobial molecules, reactive oxygen species and toxic by-products of biochemical degradation pathways. Efflux pumps can be specific for only one substrate or can export a range of molecules. The presence of efflux pumps contributes to the maintenance of cell viability, virulence and quorum sensing, assisting the bacterial cell in tolerating rapidly changing environments.

This mechanism of antibiotic resistance is responsible for reducing the intracellular concentration of the antimicrobial to a sub-inhibitory concentration, at which other more robust and specific resistance mechanisms evolve and render the antimicrobial ineffective.

Six families of efflux pumps have been described in the literature as having multidrug efflux capacity, of which the resistance/nodulation/cell division family (RND) family is most relevant in β-lactam resistance (Table 4).

Table 4. *Members of the RND efflux pump with relevance in* P. aeruginosa *(adapted from (Masuda* et al *2000; Lorusso* et al *2022)*

Examples of efflux pump	Examples of substrate
MexAB-OprM	β-lactams except imipenem
MexXY-OprM	β-lactams except ceftazidime and meropenem
MexCD-OprJ	β-lactams except ceftazidime and meropenem

The genes that encode these systems are under the control of different regulatory factors, so the expression levels of these systems vary under different conditions. For example, MexAB-OprM displays a constitutive expression and is capable of expelling the broadest range of antimicrobials, offering a protective base level production of the system to consistently expel a wide range of molecules (Li *et al* 2015). MexXY-OprM on the other hand is inducibly expressed (Thacharodi and Lamont 2022) leading to hyperproduction and eventual resistance to cefepime and other classes of antibiotics such as aminoglycosides (Kothari *et al* 2023).

Enzymes

P. aeruginosa harbors a chromosomal drug-inducible gene, blaAmpC, encoding a wide-spectrum class C β-lactamase (AmpC). The hyperproduction of this intrinsic inducible cephalosporinase is the main mechanism used by *P. aeruginosa* to resist β-lactams. AmpC induction starts with a β-lactam antibiotic increasing cell-wall degradation product in the bacterial cytoplasm. These degradation products form a complex with the regulator AmpR. AmpR is an inhibitor of AmpC production, so when it is complexed with the cell wall products, it is not available to continue repressing AmpC production. AmpC production is thereby de-repressed (Torrens *et al* 2019).

Although this comes at a fitness cost to bacteria because of the cytoplasmic accumulation of degradation products, AmpC hyperproduction will be maintained if the β-lactam antibiotic exposure continues. Exposure to β-lactams can trigger a cascade of events leading to significant AmpC production and β-lactam resistance, even for infections caused by initially susceptible isolates. The risk of inducing AmpC production varies by β-lactam (Figure 2).

Piperacillin-tazobactam, aztreonam, and third-generation cephalosporins (e.g. ceftriaxone) are weak inducers of AmpC hyperproduction but can be hydrolysed if enough β-lactamase is made. Cefepime, a fourth generation cephalosporin is a weak inducer but at the same time withstanding hydrolysis by AmpC β-lactamases because of the formation of a stable enzyme complex (Tamma *et al* 2019; López-Argüello *et al* 2021).

Acquired resistance

Horizontal gene transfer

Horizontal gene transfer (HGT) is an important source of genetic diversity and plays an important role in bacterial evolution. It frequently occurs via mobile genetic elements (MGEs). MGE is a generic term for a variety of genomic sequences such as plasmids, transposons, insertion sequences and phages. They share the ability to be transferred and are able to move within the host genome as well as jump across genomes, changing and evolving with chromosomal genomes. MGEs can change their location, copy number, provide new functions or affect chromosomal gene expression. They facilitate gene gain and loss, and have a profound effect on bacterial fitness, which contributes to genetic adaptation to new environments or conditions.

Some MGEs integrate into the chromosome in regions of genomic plasticity. Plasticity refers to the ability of certain regions within the genome to be receptive to foreign DNA, resulting in a genome size that can range from 5.5 million base pairs (Mb) to 7 Mb (Schmidt *et al* 1996). This makes it significantly larger than the genome of other bacteria such as Escherichia coli whose genome is between 4.5 and 5.5 Mb (Rode *et al* 1999) . The variation in size of the genome reflects the variability of its accessory component, which can comprise up to one fifth of the total genome of *P. aeruginosa*. MGEs may encode for alternate metabolic pathways or confer antimicrobial resistance.

Modern phylogenetic analyses have estimated the age of serine β-lactamases to be more than 2 billion years old (Hall and Barlow 2004) with plasmid-encoded β-lactamases appearing millions of years ago (Barlow and Hall 2002; D'Costa *et al* 2011; Bush and Bradford 2020) well before the advent of antibiotics. The fact that most of the virulence determinants and several antibiotic resistance genes of *P. aeruginosa* are usually present in their core genome supports the theory that these elements have evolved to deal with functions other than infecting humans and evading antibiotics (Sanz-García *et al* 2021).

Transfer events are likely to take place in dense multispecies communities such as the human intestinal tract, supplying potential pathogens with a wide array of antibiotic resistance factors (Kent *et al* 2020; Anthony *et al* 2021; Lamberte and Van Schaik 2022).

Figure 2. *Inducers of AmpC production, from strong to weak (Jones* et al *1997)* .

Table 5. The Ambler classification system

Ambler Class	Used for substrate hydrolysis	Examples of enzyme	Type of enzyme
A	serine	TEM, SHV, GES, CTX-M, KPC	Narrow-spectrum β-lactamases, Extended-spectrum β-lactamases, carbapenemases
B	a bivalent metal ion, usually zinc	IMP, VIM, NDM	Metallo β-lactamases
C	serine	AMPC, CMY	Cephalosporinases
D	serine	OXA-48 like, OXA-23	OXA-type β-lactamases

β-lactamases are commonly classified according to one of two systems – Ambler (molecular classification is based on the amino acid sequence of the enzymes), and Bush (functional classification that takes into account substrate and inhibitor profiles). Of the two, the Ambler system is more widely used (Table 5).

There are geographic preferences for specific variants, however worldwide the most frequently reported ESBLs in *P. aeruginosa* include those in Class D and Class A, while Class B (e.g. NDM and IMP) are the most prevalent carbapenemases.

The prevalence of KPC carbapenemases worldwide remains low, but are present more frequently in the Americas, the Mediterranean countries and China, whereas NDM metallo-β-lactamases are more prevalent in the Indian subcontinent and Eastern Europe (Bush and Bradford 2020). In Australia, IMP and NDM are the most prevalent (Reyes *et al* 2023).

The link between high risk clones and horizontally acquired resistance mechanisms is well-established, as is a significant relationship between high risk clones and mutational resistance, with most resistant *P. aeruginosa* isolates belonging to just a few clones (Horcajada *et al* 2019).

Table 6. Mutational resistance mechanisms of P. aeruginosa *(Oliver* et al *2023)*

Agent	Main mutational resistance mechanism	Alternative mutational resistance mechanism
piperacillin/tazobactam, ceftazidime	AmpC hyperproduction	PBP3 alteration, GalU inactivation
cefepime, aztreonam	MexXY-OprM efflux pump upregulation, AmpC hyperproduction	PBP3 alteration, GalU inactivation
meropenem	Reduction of OprD expression, MexAB-OprM efflux pump upregulation	PBP3 alteration, GalU inactivation
ceftolozane/tazobactam	AmpC structural mutation	PBP3, GalU inactivation, upregulation of efflux pumps
ceftazidime/avibactam	AmpC structural mutation, MexAB-OprM efflux pump upregulation	PBP3 alteration, GalU inactivation
imipenem/relebactam*	Reduction of OprD expression	MexAB-OprM efflux pump upregulation, PBP2, PBP1a
meropenem/vaborbactam*	Reduction of OprD expression, MexAB-OprM efflux pump upregulation	PBP3, GalU
cefiderocol*	Iron transporters, AmpC structural mutation	PBP3, galU

**Not yet approved by Australian Register of Therapeutic Goods; available in Australia via the Special Access Scheme only.*

Mutations

Mutations in specific genes or their regulators that produce or enhance resistance emerge as a result of selection pressure from antibiotics, disinfectants or other environmental pressures. Mutations in chromosomal genes leading to resistance are known as the 'mutational resistome' (López-Causapé *et al* 2018). The advent of whole genome sequencing (WGS) has allowed the mutational resistome to be studied.

Mutations in PBPs that occur regularly in Gram-positive bacteria have also been shown to occur in *P. aeruginosa* (Glen and Lamont 2021). For example mutations in the ftsI gene, which encodes PBP3 in *P. aeruginosa*, appears to have a significant role in β-lactam resistance (Del Barrio-Tofiño *et al* 2017).

The galU gene codes for an enzyme involved in the synthesis of the lipopolysaccharide core. Mutations in galU result in inactivation of the protein GalU (uridyltransferase) and this causes increases in the minimum inhibitory concentrations (MICs) of ceftazidime and meropenem (Oliver *et al* 2023).

Table 6 shows the main mutation mechanism involved in resistance to various anti-pseudomonal antibiotics.

Fitness costs

The acquisition of MGEs and mutations can come with associated fitness costs. Direct effects include the cost of the transfer process itself, the disruption of the bacterial genome by the integration of foreign DNA and the metabolic costs associated with the replication, and more importantly the expression of the newly acquired genes. Indirect effects arise mainly from the interaction between the proteins encoded by the mobile genetic element and the host, which can lead to the disturbance of cellular networks and cytotoxic effects (San Millan and MacLean 2017). Chromosomal resistance mutations carry a larger cost than acquiring resistance via a plasmid, which may be why acquired resistance is more common than mutation driven resistance (Vogwill and MacLean 2015).

While there are some resistant high risk clones that maintain high virulence despite their resistance profile, a common theory is that mutations that confer antibiotic resistance may reduce the fitness of the bacteria in the absence of antibiotics, allowing resistant mutants to be outcompeted by antibiotic-susceptible bacteria (Olivares Pacheco *et al* 2017; Glen and Lamont 2021). This may explain why a resistant strain may revert to susceptible in the absence of antibiotics a phenomenon referred to as phenotypic reversion(Dunai *et al* 2019; Liu *et al* 2022).

Antibiotics apply selection pressure

The correlation between antimicrobial consumption and antimicrobial resistance has been drawn convincingly in a number of studies (Mladenovic-Antic *et al* 2016; Martínez-García *et al* 2018; Abbara *et al* 2019; Pettigrew *et al* 2019). Analysis of global data supports the idea that reduced antimicrobial consumption is strongly associated with reduced antimicrobial resistance rates (Ajulo and Awosile 2024). One study specifically showed that prior exposure to carbapenems was an independent risk factor for blood stream infection caused by carbapenem resistant *P. aeruginosa* (Wei *et al* 2023).

Another study published in 2023 by Diaz Caballero *et al* challenged the view that infections are caused by a single genetic clone of bacteria, and that resistance evolves because new genetic mutations that are resistant to that treatment occur during the infection. The results of the study suggest that infections are in fact caused by multiple clones of a pathogen, and that resistance emerges as the susceptible organisms are killed off by the antibiotic and the organisms which possess a resistance mechanism survive and proliferate (Diaz Caballero *et al* 2023). Another theory is that the susceptible clone is replaced by a resistant clone that is a component of the patient's own microbiome (Cohen *et al* 2017). It is perhaps a high level of within-host diversity that explains why *P. aeruginosa* rapidly adapts to antibiotic treatment.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) is performed to assess the ability of an antimicrobial agent to inhibit or kill an organism in vitro. AST is a critical function of the clinical microbiology laboratory. The essential goals of AST are to guide therapy decisions, influence stewardship policy and to monitor the emergence of resistance in organisms.

AST can also help to identify isolates with defined resistance mechanisms of major interest to infection prevention and control (for example carbapenemase-producing *P. aeruginosa*). AST is furthermore the key for the assessment of incidence and prevalence in epidemiological studies that examine the origin and spread of resistance during an outbreak, including studies on the effectiveness of measures taken to counteract spread.

Data about susceptibility and resistance can be collected and used to collate information on local patterns and trends and this information allows antibiograms to be generated. Antibiograms are tables that summarise the proportions of pathogenic organisms that are susceptible to particular antimicrobials. This provides a profile of the susceptibilities of specific pathogenic bacteria

to antimicrobial agents as tested in routine clinical microbiology practice. The use of cumulative antibiograms is an aid to antimicrobial stewardship (AMS) programs in the development of local antimicrobial prescribing guidelines and formulary management (Australian Commission on Safety and Quality in Health Care 2019).

This information is used together with therapeutic guidelines when creating policies guiding the choice of empiric therapy. There is evidence to suggest that the earlier a patient receives appropriate treatment for an infection, the better the outcome (Raman *et al* 2018; Bassetti *et al* 2020; Zasowski *et al* 2020) and as AST will take at least one or two days, these policies provide an evidence-based guide to antibiotic choice that is fitting for local resistance trends.

AST is typically performed by determining the MIC and interpreting this based on defined breakpoints. Broth microdilution is the gold standard (or agar dilution for some organism/drug combinations) and disc diffusion and gradient tests such as Epsilometer testing (E-tests) are derivative methods that are calibrated to the reference method. Automated susceptibility testing platforms such as Vitek (bioMe'rieux, Marcy-l'Étoile, France) and Phoenix (BD Diagnostics, Franklin Lakes, New Jersey, USA) are also in common use.

Breakpoints for phenotypic antimicrobial susceptibility testing are determined by the development laboratory by taking into account:

- knowledge of dosage
- modes of administration
- target infections
- clinical outcome when wild type organisms of defined species are treated
- pharmacokinetics and pharmacodynamics of the agent
- MIC distributions and epidemiological cut-off values (ECOFFs)
- resistance mechanisms
- zone diameter distributions

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) breakpoints are the two more commonly used guides for interpretation. Calibrated Dichotomous Sensitivity Test (CDS) is also in use in laboratories around Australia but will cease being supported at the end of 2025.

Interpretations are typically abbreviated to 'S', 'I' and 'R'. While 'S' and 'R' are defined as being susceptible and resistant by both EUCAST and CLSI, the 'I' category is defined as being intermediate by CLSI, but as 'susceptible, increased exposure' by EUCAST. The EUCAST 'I' is to indicate that therapeutic success is likely when there is increased exposure either by way of adjusting dosing, or by virtue of high concentration of an antibiotic at the site of infection. For *P. aeruginosa* there is no longer an 'S' categorisation for multiple antibiotics including ceftazidime, cefepime, piperacillin-tazobactam, and aztreonam. For these antibiotics there is an 'I' category, indicating that the isolate will need increased exposure (adjusted dosing) for successful treatment.

Once an organism has been isolated, identified and its significance in causing an infection established, susceptibility testing can be performed to provide the clinician with guidance in choosing an antibiotic that is likely to be effective in treating the infection caused by that organism.

A range of antimicrobials is routinely tested, based on the identification of the organism isolated, the site of infection and the availability of the antibiotic in the hospital (i.e. the formulary). Both EUCAST and CLSI include recommendations for agents that are important to test routinely as well as expected intrinsic resistance. The hospital may have also developed their own set of local guidelines, reflective of the range of resistance they encounter. The range of antimicrobials that is tested includes members of the different antibiotic classes to maximise the probability of identifying an antibiotic that will be effective against the organism, enable detection of resistance mechanisms, ideally contain both oral and intravenous options and offer alternatives in case of allergy or other reason a patient cannot have the antimicrobial administered (e.g. impaired renal function).

The panel of antibiotics will include first line agents as well as some alternative second line agents. An AMS strategy in common use in Australia is selective (or cascade) reporting, where many agents are tested, but some results are not reported unless the first line agents are resistant. National guidelines were set up for Australian reporting by the Royal College of Pathologists of Australia in 2019, then updated in 2021(The Royal College of Pathologists of Australiasia 2021). Selective reporting has been shown repeatedly to influence the prescribing of antibiotics (Katchanov *et al* 2017; Pulcini *et al* 2017; Langford *et al* 2019; Liao *et al* 2020; Tebano *et al* 2020; Kahlmeter *et al* 2021) and is an important AMS tool. In sites where resistance is widespread, a longer list of agents that includes novel β-lactam/β-lactam inhibitor agents may be needed. The most recent Infectious Diseases Society of America guidance document strongly encourages all clinical microbiology laboratories to perform AST for MDR and DTR-*P. aeruginosa* isolates against novel β-lactam agents i.e. ceftolozane-tazobactam, ceftazidime-avibactam, and cefiderocol (Tamma *et al* 2023) however in

Australia where resistance is less prevalent, these agents may only be tested if the isolate proves to be resistant to other agents.

Most laboratories will not have the resources nor trained staff to perform gold standard microbroth dilution but should be able to perform AST by derivative methods such as disc diffusion, E-tests or by an automated broth dilution method for example Vitek or Phoenix.

Detection of resistance in the laboratory

Phenotypic testing refers to growth-based methods and provides information on which antimicrobial will either kill or arrest growth of an organism, whereas genotypic AST attempts to identify specific resistance genes or genetic mutations using molecular or genomic (usually DNA-based, amplification-based or sequencing-based) methods.

Genotypic testing can be performed in minutes. Conversely, phenotypic AST usually requires at least overnight incubation (sometimes longer) and may not always accurately reflect the underlying genotype of a microorganism, meaning additional testing may be required.

Figure 3 is an image of phenotypic AST by disc diffusion of *P. aeruginosa*. A lawn culture of organism at a known concentration was inoculated onto the plates and filter paper discs impregnated with different antibiotics were applied. The plates were then incubated overnight. A zone of no growth appears around the discs containing antibiotic that has either killed or arrested the growth of the *P. aeruginosa*. These zones are measured and compared to breakpoint tables to help decide whether the organism is susceptible, resistant or otherwise to each antibiotic:

Measuring zone sizes and using EUCAST breakpoints, this result reveals:

- This isolate is susceptible to tobramycin and amikacin (TOB and AK)
- This isolate is resistant to piperacillin/tazobactam (TZP), aztreonam (ATM), ceftazidime (CAZ), imipenem (IPM), cefepime (FEP), ciprofloxacin (CIP) and meropenem (MEM). (There is no interpretive breakpoint for gentamicin (CN).)
- Further testing with some of the newer β-lactam/β-lactamase inhibitor combinations for example ceftazidime/avibactam and ceftolozane/tazobactam may be clinically useful and should be performed if requested by the clinician.
- This pattern of resistance (resistant to all β-lactams tested including carbapenems) may indicate the presence of a carbapenemase, and investigation for this is warranted for infection prevention and epidemiological purposes.

The antibiogram of this isolate does not necessarily reflect the production of carbapenemases, as other mechanisms can also cause this pattern of resistance. Since carbapenemase-encoding genes are often located on MGEs, this type of resistance is capable of spreading quickly in a healthcare setting. Therefore, distinction between carbapenem-resistance mediated by transmissible carbapenemases and resistance mediated by other mechanisms is important for infection control.

Figure 3. *AST testing of* P. aeruginosa *by disc diffusion*

Additionally, clinical outcome was found to be worse in patients with carbapenemase-producing *P. aeruginosa* infections compared with non-carbapenemase-producing *P. aeruginosa* infections, even after adjusting for a number of factors (Reyes *et al* 2023).

Investigation for carbapenemase can involve phenotypic testing to detect the presence of an enzyme, but ultimately genotypic testing will be required to identify the genes present.

Phenotypic testing for carbapenemases

The modified Hodge Test - carbapenemase activity is detected when the clinical isolate producing carbapenemase allows growth of a strain towards an imipenem disc. While low cost and simple to perform, this test is prone to false positives (due to AmpC production) and false negatives (e.g. NDM-producing isolates) and has largely been superseded by more reliable methods.

The carbapenemase inactivation method (CIM) is based on the enzymatic hydrolysis of a meropenem disc after its exposure to a carbapenemase producing strain and its consequent inactivation which allows uninhibited growth of a fully susceptible indicator strain. It is also low cost and easy to perform using reagents most microbiology laboratories would already have on hand. Developed in 2015, with reportedly good sensitivity and specificity (Van Der Zwaluw *et al* 2015), a modified method (mCIM) was developed and increased sensitivity and specificity for *P. aeruginosa* to 98% and 95% respectively (Simner *et al* 2018). Yet another modification was made (CIMTris), in which carbapenemase is extracted from bacteria with Tris-HCl buffer, which further increased the sensitivity and specificity for *P. aeruginosa* to 99.3% and 95.0% respectively (Uechi *et al* 2019). One disadvantage to CIM testing is that it requires overnight incubation.

Carba NP test is a colorimetric assay based on the enzymatic hydrolysis of the β-lactam ring of a carbapenem leading to a pH decrease and consequent colour shift of the pH indicator in the presence of carbapenemase activity. This test and its variants require the acquisition of dedicated reagents (with associated costs and training needs), are interpreted subjectively and have poor sensitivity for both mucoid isolates and for the detection of OXA-48 type carbapenemases (Tamma and Simner 2018). It offers the advantages of reduced time to result, commercial availability of kits as well as discrimination between the different carbapenemases. The subsequently developed NitroSpeed Carba NP test (Nordmann *et al* 2020) has improved sensitivity and specificity for *P. aeruginosa* to 100% and 100% respectively by increasing the incubation time to 40 minutes (Wang *et al* 2021).

Occasionally false positive phenotypic tests are reported for *P. aeruginosa*. This is where the phenotypic test is positive, but no carbapenemase gene is identified using genotypic methods. Possible reasons for this are hyperproduction of AmpC or rarely due to the presence of a novel carbapenemase gene (Van Der Zwaluw *et al* 2015).

Matrix assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF), commonly used in laboratories to identify organisms can also be used to identify cabapenemase production. Two approaches have been followed with one detecting carbapenem degradation products and the other detecting a known carbapenemase-bearing plasmid-associated protein peak. Using the MALDI-TOF for this purpose however requires different settings to those used for identifying organisms.

Lateral flow immunoassays have been developed to detect carbapenemases. While the early ones allowed the detection of only one carbapenemase at a time, a multiplex assay has been developed (Boutal *et al* 2018) and it compares well to genotypic methods (Nishida *et al* 2022). The cost per test is about half that of GenXpert Carba-R (Sullivan *et al* 2023) with the added benefits of no outlay for instruments, no special expertise required and faster time to result.

Genotypic testing for carbapenemases

Few bacteriology laboratories are equipped to perform genetic testing of isolates. Some larger hospitals may have molecular biology departments with instruments such as COBAS (Roche Diagnostics, Basel, Switzerland), Alinity M (Abbott, Illinois, United States) or Alliance (AusDiagnostics, Sydney, Australia) that are capable of running commercial kits developed to detect carbapenemase genes. In the absence of separate molecular departments, smaller instruments such as the Cepheid GeneXpert or BD Max enable the entire multiplex PCR reaction to occur inside a small cartridge, obviating the need for expensive and complex instrumentation. These systems are capable of detecting carbapenemase genes both from bacterial isolates as well as directly from rectal swabs or positive blood cultures with excellent sensitivity and specificity (Table 7). These small units have enabled this sort of assay to become a point of care test (Pannala et al 2018; Ambretti et al 2019)which could remove this testing from the auspices of the laboratory.

Table 7. *Comparison of several automated real time PCR assays*

Assay	Sample type	Sensitivity	Specificity
Xpert Carba-R (Bai *et al* 2021)	Culture isolate	100%	98%
	Rectal swab	95%	99%
Film Array BCID (Peri *et al* 2022)	Positive blood culture	94.9%	99.7%
BD MAX™ CRE Assay (Girlich *et al* 2020)	Culture isolate	97.1%	98.8%
	Rectal swab	92.8%	97.8%
Entericbio CPE assay (Vanstone *et al* 2018)	Culture isolate	100%	100%

Whole genome sequencing

Whole genome sequencing (WGS) can:

- identify organisms by species and strain
- identify AMR genes and offer an AMR prediction
- be useful in outbreak recognition and subsequent investigation
- can provide information on phylogeny and transmission dynamics of organisms for infection prevention purposes.

While this is important information, the immediate requirement for the clinician treating a patient with an infection is knowledge about which antimicrobial agent is likely to be effective and which agents are not. This crucial information is provided by the local microbiology laboratory.

The complex WGS process is simplified in Figure 4.

This process can take anywhere from 1 to 7 days, and can cost $AUD150 per test (Elliott al 2021), dependent on scale and throughput, and after the initial costs of instrument acquisition and installation.

Currently WGS is usually only performed in research and reference laboratories. The extensive training required to perform this test as well as the lack of bioinformatics specialists are challenges that are difficult to overcome in implementing WGS in microbiology laboratories. The financial costs of both the capital equipment and bioinformatic and computing software are other important considerations.

The results of WGS can provide valuable information on a *P. aeruginosa* isolate that is resistant to all β-lactams. It informs about the presence of a carbapenemase (or any other AMR) gene and determines the variant of the gene. This information can be used to predict antibiotic resistance in the single isolate, as well as look for patterns in collections of isolates to detect outbreaks and determine transmission events.

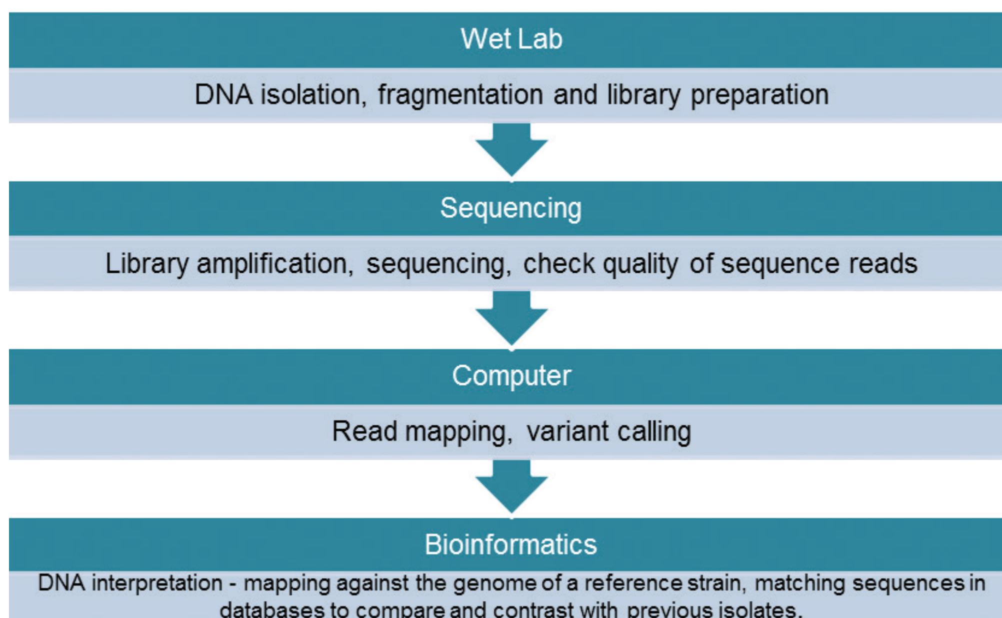

Figure 4. *Summary of whole genome sequencing process (Bagger* et al *2024)*

Surveillance, data collection and sharing of information

β-lactams are one of the older antibiotic classes and are generally well-tolerated, meaning they are suitable agents for first line therapy (Chiriac *et al* 2017). As discussed, some of the β-lactam resistance is caused by transmissible mechanisms, and are therefore avoidable with careful infection prevention strategies. Surveillance is a valuable information tool for planning actions and for monitoring the impact of interventions.

Collection of data allows the scope of a resistance problem to be defined, both geographically and by patient type. Carefully collected data can also be used for benchmarking and indicating a need for process improvement. Important requisite for benchmarking is having this information publicly available and properly resourced ie government funded as opposed to funded by the corporate sector (Núñez-Núñez *et al* 2018). Additionally, information needs to be sufficiently detailed to be useful, so the data collection must be in a format that allows optimised analysis of the data with regard to factors such as hospital versus community acquired infection and any identified risk factors for infection.

Objective number five of Australia's National AMR Strategy (Commonwealth of Australia (Department of Health), 2019) concerns an integrated response to antibiotic resistance as well as usage. Various surveillance initiatives contribute to this national system, called the Antimicrobial Use and Resistance in Australia (AURA). It is government funded, and the organisms included are considered to be a priority for surveillance of antimicrobial resistance in Australia. *P. aeruginosa* is included as a Priority Set 2 organism – where the impact of resistance is substantial in hospital settings.

Four key long-term programs that contribute are:

1. AGAR – Australian Group on Antimicrobial Resistance – around 40 laboratories provide data and samples on 3 groups of bacteria from blood stream infections (Staphylococci, Enterococci and Gram-negative rods). This information is published in a combined report by the Australian Commission on Safety and Quality in Health Care (ACSQHC).

2. NAUSP – National Antimicrobial Utilisation Surveillance Program – established by SA Health, antimicrobial usage data is provided voluntarily from public and private hospitals from around the country (currently over 230 sites). This enables participant hospitals to benchmark their usage to that of other sites, and highlights changes or overuse of agents.

3. NAPS – National Antimicrobial Prescribing Survey – run by a team from the Royal Melbourne Hospital and the National Centre for Antimicrobial Stewardship at the Doherty Institute, it collects data about antimicrobial prescribing by Australian Hospitals and aged care facilities via online survey.

4. APAS – Australian Passive AMR Surveillance – provides the largest volume of surveillance data to AURA on de-identified patient-level AMR data. This data provides access to trends in resistance at both organism and geographical levels. ACSQHC established this system in 2012, using the Queensland Health OrgTRx System as the IT infrastructure. Private and public pathology services across Australia contribute data voluntarily.

AURA also relies on data from other programs such as the National Alert System for Critical Antimicrobial Resistances (CARAlert). It collects data on nationally agreed priority organisms with critical resistances (e.g. carbapenemase producing *P. aeruginosa*) that are known to present a serious threat to the effectiveness of last-line antimicrobials. Established by ACSQHC in 2016, it obtains data from public and private health services across Australia voluntarily via an online portal and publishes reports bimonthly.

Data collected in Australia contributes to global data such as the World Health Organisation's Global Antimicrobial Resistance and Use Surveillance System (GLASS). Data from GLASS helps inform national, regional and global decision making. It is also an invaluable tool that can be used in the care of patients with a history of overseas travel. The emergence of resistance in one part of the world can potentially become a problem in another location due to travel of humans around the world and dissemination of resistant strains of organisms (Frost *et al* 2019; Berndtson 2020). With reports only being published annually, this means there is a delay in conveying important information. A move to real time reporting would certainly add value to these surveillance programs.

Conclusion

The evidence that many of the resistance mechanisms of *P. aeruginosa* have been present in the species well before the advent of antibiotics supports the idea that this is a versatile and resilient organism that will continue to adapt to whichever environment it finds itself in.

While new antimicrobial agents and testing strategies develop reactively to the discovery of new resistance mechanisms, areas where we can take immediate action are preventing the spread of transmissible resistance mechanisms, more judicious use of the antibiotics we have available and more efficient dissemination of surveillance data.

The microbiology laboratory continues to have an important role in the detection and surveillance of resistance and remains an essential partner of antimicrobial stewardship and infection prevention activities in efforts to combat resistance in *P. aeruginosa*.

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ORIGINAL ARTICLE

Comparison of laboratory coagulation tests and thromboelastography and their influence on transfusion algorithms in cardiac surgery

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Fellowship Dissertation

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Abstract

Thromboelastography (TEG) provides information on the dynamics of clot development, stabilisation and breakdown that reflect *in vivo* haemostasis. TEG is being increasingly used as a Point of Care (POC) test to direct transfusion management in cardiac surgery (Ortmann *et al* 2015; Karkouti *et al* 2016) and has been well documented to show reduced blood product usage associated with better patient outcomes (Shore-Lesserson *et al* 1999, Fleming *et al* 2017; Redfern *et al* 2019; Tyler *et al* 2021). Laboratory coagulation testing is also used in decision making for transfusion requirements during and after surgery. This study aims to describe the relationship between TEG parameters and the corresponding laboratory coagulation tests and then compare those parameters for the Monash TEG-guided Perioperative Algorithm which is used to direct transfusion in patients with clinically significant bleeding.

A retrospective study was conducted at the Victorian Heart Hospital (VHH) utilizing data from its inaugural year of operation. The patient cohort included patients that had a Massive Transfusion Protocol (MTP) activated or required Emergency Coagulation Packs whilst having cardiac surgery. TEG parameters were compared with laboratory coagulation tests to determine the relationship between the two modes of testing. Measures of clot initiation, clot formation and clot strength were examined using the TEG® 6s – a 4 channel POC analyser that includes Normal TEG, Rapid TEG, Heparinase TEG and Functional Fibrinogen TEG. The laboratory tests performed were internationalized normal ratio (INR), activated partial thromboplastin time (APTT), fibrinogen and platelet count. Correlations between various TEG parameters and the standard laboaratory tests were analysed and the TEG- guided Perioperative Algorithm used at VHH was then examined by comparing Rapid TEG to platelet count, Heparinase TEG to INR and Functional Fibrinogen TEG to fibrinogen to identify how these relationships affect transfusion of blood products.

Keywords: ***thromboelastography, coagulation, cardiac surgery, transfusion***

Introduction

The Victorian Heart Hospital (VHH) is Australia's first dedicated cardiac hospital, combining clinical cardiology services, research and education. It is one of seven Monash Health hospitals, Victoria's largest public health service. VHH received its first patients in February 2023 and once fully operational, will provide over 2,000 open heart surgeries annually. Cardiac surgeries, particularly those requiring the use of cardiopulmonary bypass (CBP), often result in perioperative bleeding requiring transfusion of blood products (Meco *et al* 2020) with the incidence of coagulopathy in critically ill cardiac patients around 30% (Shen *et al* 2017; Keri 2023). Current literature comparing TEG and laboratory testing most commonly study the cardiac patient population where surgeons and anaesthetists need to manage both CBP and dilutional coagulopathies (Cohen *et al* 2020). At Monash Health, patients with clinically significant surgical bleeding are monitored using both TEG and laboratory coagulation tests. There are three TEG analysers used across two Monash sites, one at Monash Medical Centre, Clayton and two at VHH. VHH also has a 24-hour on-site pathology service with two laboratory coagulation analysers (Werfen ACLTOP 350) and two haematology analysers (Beckman Coulter DxH690T) in use. The clotting tests performed at VHH include INR, APTT, fibrinogen, TCT and D-Dimer. Full blood examinations (FBEs) are performed on the DxH690 to provide haemoglobin and platelet counts which are used in the Perioperative Algorithm to guide transfusion of blood products.

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The use of TEG-guided algorithms in cardiac surgery has been well documented and shown to reduce transfusion needs and improve patient outcomes (Fleming *et al* 2017; Redfern *et al* 2019). Laboratory coagulation testing is also routinely performed on cardiac surgery patients but due to the longer turn-around-time (TAT), these results may not be as useful in decision making on immediate transfusion requirements. TEG and laboratory tests both measure aspects of the clotting process but on different types of patient samples. The TEG measures clotting on a whole blood sample collected in a citrate tube whilst the laboratory tests are performed on citrated plasma after the sample has been centrifuged. There have been several studies comparing TEG and coagulation test results with varying levels of consistency in their associations (Wang *et al* 2018; Cohen *et al* 2020; Lloyd- Donald *et al* 2020). The aim of this study was to first describe the relationship between TEG parameters and corresponding laboratory coagulation tests in a group of cardiac surgery patients at VHH. Those parameters used in the TEG-guided Perioperative Algorithm to assist decision making in the treatment of patients with clinically significant bleeding were then compared.

Materials and methods

Patient selection

Monash Pathology at VHH opened on March 8th 2023. The patients examined in the study included those that received transfusion of blood products between March 8th 2023 and March 8th 2024 in two categories: MTP patients and those receiving Emergency Coagulation Packs.

According to ANZBT guidelines, a massive transfusion is defined as either transfusion (in an adult person) of more than one blood volume (i.e. 10 units in 24h) or in acute situations transfusion of half the blood volume (five units) in 4 h. An MTP is activated when there is actual or anticipated transfusion of four units of RBC in < 4 h and the patient is haemodynamically unstable with or without ongoing bleeding. For a cardiac patient, MTP packs are issued in rounds as follows:

Round 1 = 4 units RBC/2 units FFP/1 unit platelets

Round 2 = 4 units RBC/2 units FFP/5 units cryoprecipitate

Round 3 = 4 units RBC/2 units FFP/5 units cryoprecipitate /1 unit platelets Then alternating Rounds 2 and 3 if further products are required.

Cardiothoracic surgery patients requiring Emergency Coagulation Packs were the second group of patients examined. Cardiac patients are often on CBP with cell salvage to reduce the need for red cell transfusion. Cell salvage is a method of harvesting red cells lost during surgery, processing then preparing them for safe return to the patient's own circulation. Intraoperative cell salvage may be preferable to donor transfusion in the operative setting (Carroll *et al* 2020) as cell salvage can provide red cell requirements with autologous red cells. Emergency Coagulation Packs are helpful during or immediately after surgery for patients who require coagulation factors but not additional red cells. At Monash, the laboratory protocol recommends that one hour before CBP ends the clinical team notifies the Blood Bank that an Emergency Coagulation Pack will be required consisting of 4 units FFP/2 units platelets /10 units cryoprecipitate. If the patient continues to bleed a second Emergency Coagulation Pack may be requested. Depending on the results of the TEG, the specifications of the second Emergency Coagulation Pack may be modified by the treating team. The Blood Bank scientist then prepares a mix of coagulation products as specified by the anaesthetist. If red cell transfusion becomes necessary, an MTP may be activated.

According to the Monash TEG guided Perioperative algorithm, RBC transfusion is required if Hb <70g/L. MTP should be activated if appropriate (>4 RBC with active/ ongoing bleeding). A TEG should then be performed and an urgent FBE, INR, APTTand fibrinogen should be considered. Both groups of MTP patients and patients requiring Emergency Coagulation Packs were analysed for this study as these patients represent a cohort likely to have had a TEG test and coagulation tests performed.

TEG

TEG uses the principle of viscoelasticity to measure coagulation initiation, clot formation, clot strength and fibrinolysis. There are five different points of measurement in the TEG profile which indicate specific parameters of patient haemostasis. These parameters are R time, K time, alpha angle, maximum amplitude (MA) and LY30. The R time is a measure of the time it takes for the clot to be formed, the K time represents the kinetics of clot formation, the alpha angle is the slope between R and K, the MA indicates strength/firmness of the clot, and stability of the clot or fibrinloysis/breakdown is measured by the percent lysis after 30 minutes (LY30%). As well as the standard normal TEG parameters the TEG® 6s (Haemonetics) also has three channels that give extra information. There is a Heparinase TEG (CKH) that assesses the effect of heparin, a Rapid TEG (CRT) that gives a quick assessment of clot strength without the initiation stage and a Functional Fibrinogen TEG (CFF) that shows clot strength based on fibrinogen concentration.

The TEG parameters of interest for the current study included CK (Citrated Kaolin)- Normal TEG: R time, K time, alpha angle and MA. The TEG® 6s system consists of an analyser and disposable assay cartridges (Figure 1).

The assay cartridge contains all components necessary to allow the analyser to prepare samples and perform haemostasis tests (Figure 2). To perform a TEG analysis, a fresh blood sample is collected via venipuncture into a tube with 3.2% buffered sodium citrate at a citrate-to-blood ratio of 1:9, (the same type of sample required for laboratory coagulation tests). A pipette is used to add whole blood to the cartridge sample port, filling up to the line marked on the cartridge, then the analyser draws the blood into the active area of the cartridge and mixes it with the reagents in the cartridge. The harmonic motion of the drop of blood is measured in response to external vibration. As the sample transitions from a liquid state to a solid state during clotting, the elasticity and resonant frequency increase. These variations in resonant frequency during clotting and lysis are recorded and the results are displayed in a table and on a graphical trace that reflects clot formation shown in Figure 3 (TEG 6S User Manual 2015).

The resulting haemostasis profile is a measure of the time it takes for the first clot to be formed, the kinetics of clot formation, the strength of the clot, and the breakdown of the clot, or fibrinolysis.

Individual points in the profile indicate specific parameters of patient haemostasis. These parameters are R time, K time, Alpha Angle, Maximum Amplitude (MA), and LY30 and are indicated in the diagram below (Figure 3).

Test results are plotted on a continuously updated results screen and on a graph (tracing) to allow visual assessment of parameters. Numerical parameters are determined over the course of a test (which can run for a maximum of 90 minutes). Superimposed tracings display a Y axis that indicates the amplitude (in millimetres) and an X axis that indicates the time (in minutes). Reference ranges show the maximum and minimum limits for normal results for each parameter and appear under the parameter values (Figure 4) (TEG 6S User Manual 2015).

Figure 1. *TEG® 6s haemostasis analyser - The TEG® 6s system consists of a small analyser and disposable assay cartridges. The analyser contains an interface in the form of a touch display screen. The system is designed to accept a disposable plastic cartridge, into which a blood sample is placed.*

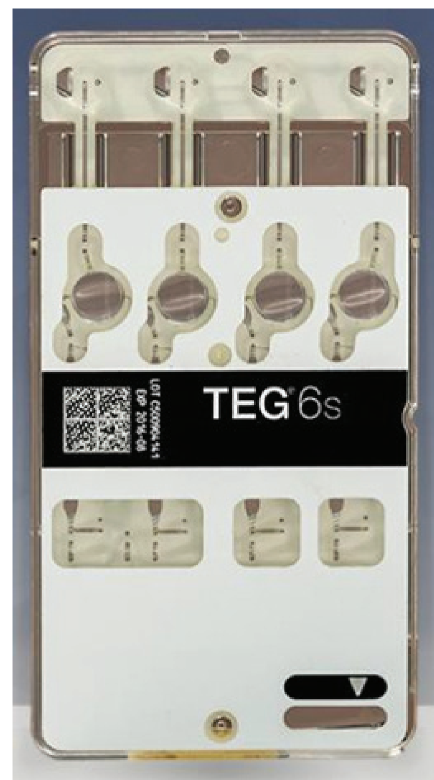


Figure 2. *TEG® 6s cartridge - There are four channels in the TEG® 6s cartridges – CK, CKH, CRT and CFF.*

CK = Citrated Kaolin - normal TEG

CKH = Citrated Kaolin Heparinase – to assess effect of heparin

CRT = Citrated rapid TEG – quick assessment of clot strength (without initiation)

CFF = Citrated Functional Fibrinogen – clot strength based on fibrinogen concentration.

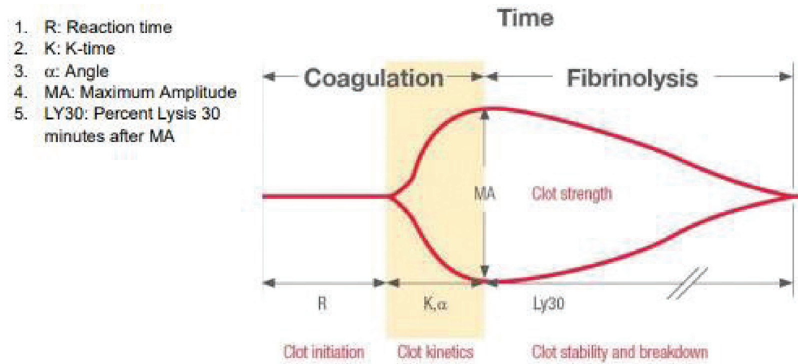


Figure 3. *TEG tracing parameters*

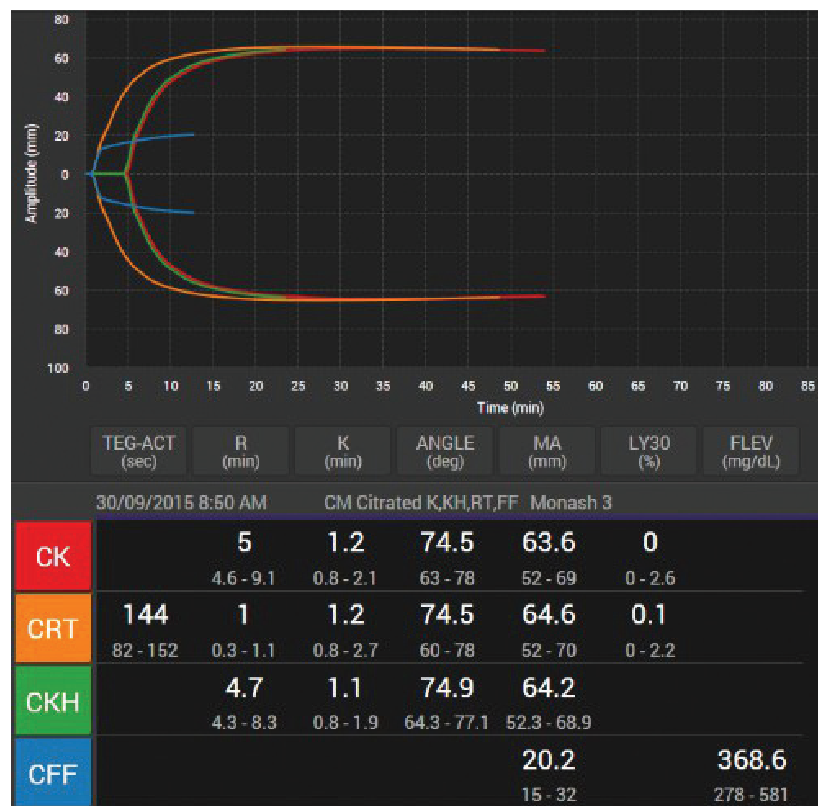


Figure 4. *Example of TEG results/trace.*

Laboratory testing

In traditional laboratory coagulation the PT/INR and APTT are the most commonly performed coagulation screening tests. The PT measures the activity of the extrinsic and common pathways of coagulation and is dependent on the functional activity of factors VII, X, V, II (prothrombin) and fibrinogen. The INR (International Normalized Ratio) is calculated from the PT and is generally used to monitor individuals who are being treated with the anticoagulation medication warfarin (Perry 2024). For the purpose of comparison, the INR has been used in this study as the standard coagulation profile at Monash Health comprises an INR, APTT and fibrinogen. The APTT measures the activity of the intrinsic of factors XII, XI, IX, VIII and common pathways of coagulation. INR and APTT are performed by recalcifying a sample of citrated plasma in the presence of tissue factor thromboplastin (PT reagent) or a negatively charged substrate (APTT reagent). This results in factor activation, thereby initiating coagulation via the different clotting pathways (Gonzalez *et al* 2010). The end point for these tests is the time (in seconds) until the formation of a fibrin clot is detected. It seemed fitting to compare TEG R times to the INR/APTT times as both tests measure the time it takes for an initial clot to form. The R time reflects the function of the coagulation factors, namely thromboplastin generation time, while the K time shows the agglutination of the red blood cells and is associated with the function of thrombin. The alpha angle represents the blood-clot formation rate with rapid fibrous-protein interaction of fibrin and platelets (Liang *et al* 2020).

The TEG alpha angles, K times and MA were compared to laboratory tests of fibrinogen and platelet count. At VHH the fibrinogen is measured via two different methods – PT derived and Clauss. For results between 2-10 g/L a derived fibrinogen is obtained by determining the PT by optical density change for a range of plasma dilutions with known fibrinogen levels. The optical change for each different fibrinogen level is then plotted as a calibration curve and the fibrinogen result is derived from the change in optical density compared to the calibration curve. For results <2g/L or >10g/L a Clauss fibrinogen is performed by adding a high concentration of thrombin (Siemens Dade Thrombin) and measuring clot formation in diluted plasma. To complete the analysis ideally a comparison of fibrinolysis would have been performed by comparing TEG LY30% and D-Dimer however due to lack of data, this was not undertaken.

The laboratory testing was conducted on ACL TOP350 (Werfen) for INR, APTT and fibrinogen. The platelet count was taken from the FBE performed on the DxH690 using impedance methodology with hydrodynamic focusing. The APTT was measured by a clot-based assay using purified phospholipid and micronized silica activator (Triniclot HS reagent, TCOAG). PT and INR were measured using platelet poor plasma collected in citrate tubes, analyzed by a clot-based assay using recombinant tissue factor in a phospholipid blend combined with calcium chloride (PT- Recombiplastin reagent, Instrumentation Laboratories). Fibrinogen was estimated using a derived fibrinogen from absorbance during the PT assay relative to a calibrator. When the derived fibrinogen was <2.0g/L, a Clauss fibrinogen was measured to give a more accurate result at this level.

Transfusion algorithm

The second part of the study involved a review of the TEG-guided Perioperative Algorithm used at Monash Health (Figure 5). There is numerous literature that supports the use of TEG algorithms in cardiac surgery showing a reduction in blood product usage and better outcomes for patients. There is currently no single standardized algorithm recommended for use in bleeding scenarios, so it appears that individual institutions adopt their own algorithm as they deem appropriate for their patient population. Some of the different patient populations that utilize TEG include trauma care (Subramanian *et al* 2014; Peng *et al* 2018)), coagulopathies of liver disease (Lloyd-Donald *et al* 2020), major surgery, obstetrics, sepsis (Kim *et al* 2021) as well as in cardiac surgery and patients on Extracorporeal Membrane Oxygenation (ECMO) (Panigada *et al* 2018; Giani *et al* 2021). Transfusion algorithms have been regularly shown to be useful for optimizing the need for blood transfusion during surgery and in the 24 h after ICU admission (Tamura *et al* 2024).

An interesting feature of the various algorithms is that they are not consistent in the TEG and/or laboratory coagulation tests that are used for decision making. The algorithm used at Monash appears below and uses the TEG parameters CFF-MA, CRT-MA and CKH-R time and/or fibrinogen level, platelet count and INR. These parameters are used to guide the transfusion of cryoprecipitate, platelets and FFP. Hb is also used to guide transfusion of red blood cells. Other institutions use the TEG parameters of CK-R, CKH-R, CFF- A10 and CRT-A10 and fibrinogen as a measure of clot strength (Figure 6) or CFF-A10, CRT-A10, CKR-CKHR and CRT-LY30 with the suggestion of repeat conventional coagulation tests (Figure 7). A third example (Figure 8) used at various other hospitals uses CFF-MA, CRT-MA, CK-R, CK-H and LY30. Another observation is the inconsistency in recommendation of volume of blood products. Some algorithms suggest quantity in relation to severity of results others suggest the product and it is left to the clinician to decide on quantity.

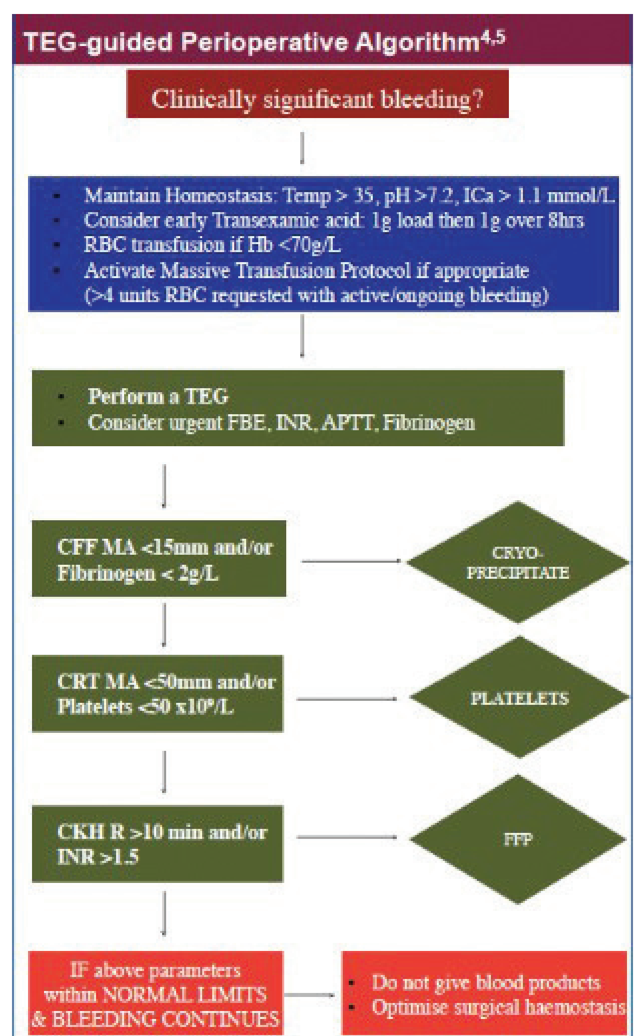


Figure 5. *Monash Health, Melbourne*

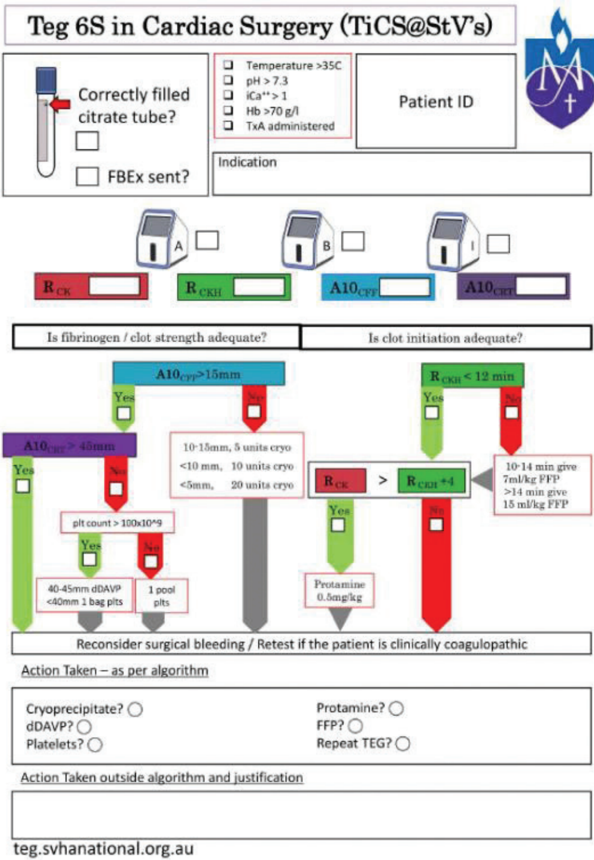


Figure 6. *St. Vincent's Hospital, Melbourne*

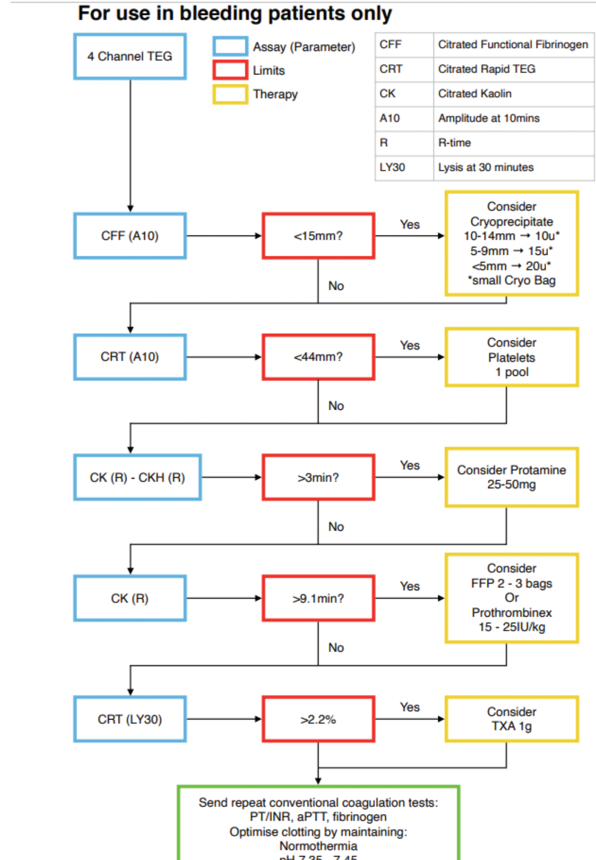


Figure 7. *Austin Hospital, Melbourne*

Figure 8. *Bendigo Health, Royal Children's Hospital, South West Healthcare - Warrnambool, Royal Hobart Hospital*

The Monash Health algorithm was used for this study. The variables of interest included CFF- MA and/or fibrinogen, to recommend the transfusion of cryoprecipitate, then CRT-MA and/or platelet count, in the same way they are reviewed before transfusing platelets. CKH-R time and INR were examined also, as these parameters are reviewed prior to the transfusion of FFP.

An analysis of the specificity and sensitivity of the TEG to predict transfusion of blood product was also undertaken and finally a case study was examined to demonstrate the TEG results, laboratory test results and blood products transfused over a 24 h period for an activated MTP. The case study demonstrated changes in the TEG and laboratory tests over time and the influence of transfusion on test results.

In summary the following comparisons were performed:

1. Coagulation initiation/clotting times = TEG CK-R times compared to INR/APTT.
2. Clot formation/kinetics = TEG CK-alpha angles & K times compared to fibrinogen levels.
3. Clot strength/firmness = TEG CK-MA compared to fibrinogen and platelet counts.
4. TEG-guided Perioperative Algorithm : A comparison of the TEG CRT-MA and platelet count, TEG CKH-R and INR and CFF-MA and fibrinogen was conducted to identify how these relationships affect transfusion of blood products.

Results

Eighty-seven adult patients were included in this study with a mean age of 64.9 years. The youngest patient was 25 y and the oldest was 83 y. Patients between 53.9 and 70.6 y made up 95% of the cohort and 31 patients were female and 56 were male. The patients were having surgical procedures for various conditions including mitral valve replacement, aortic valve replacement, coronary artery bypass grafting (CABG), aortic dissection and cardiac arrest. 163 TEG tests were run for the cohort from March 2023 to March 2024. Each of these TEG results was correlated with corresponding laboratory coagulation tests collected at the same time as the TEG or close to the TEG collection time with 65% of the coagulation tests performed within an hour of the TEG test. To assess clot initiation, TEG R times were compared to INR and APTT, to assess clot formation TEG K time and alpha angle was compared to fibrinogen level, and to assess clot strength TEG MA was compared to fibrinogen and platelet count. To examine whether the TEG parameters correlate with the laboratory coagulation results, Pearson's correlation coefficient (R value) was determined for each comparison and P values were calculated to determine correlations of significance. The data is presented as plots with linear fit ((Figs 9a-c) and the Pearson test R and P values are shown in Table 1.

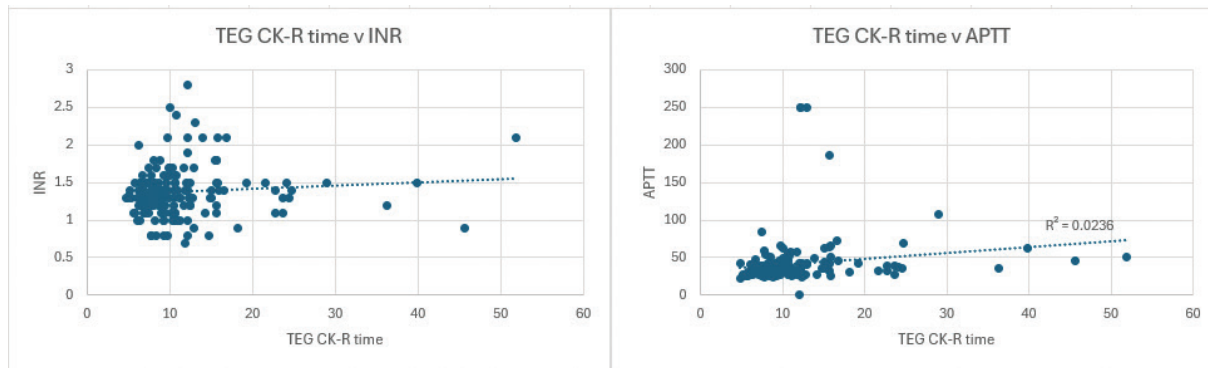
The TEG and laboratory coagulation tests show various associations with each other. The strongest correlations are shown to be between the standard TEG parameter CK- MA and platelet count (R= 0.5775, P<0.001) followed by CK-MA and fibrinogen (R=0.3933, P<0.001). The weakest correlation was between TEG CK-R times and INR (R=0.0904, P=0.2513). Other studies have also shown positive correlations between TEG MA and fibrinogen/ platelets (Liu et al 2016; Sharma et al 2018; Wang et al 2018)

The Monash TEG Perioperative Algorithm was then examined in terms of TEG parameters, laboratory coagulation tests and products transfused. The parameters analysed included: CRT-MA, platelet count and platelet administration, CKH-R, INR, APTT and FFP administration then CFF-MA, fibrinogen and cryoprecipitate administration.

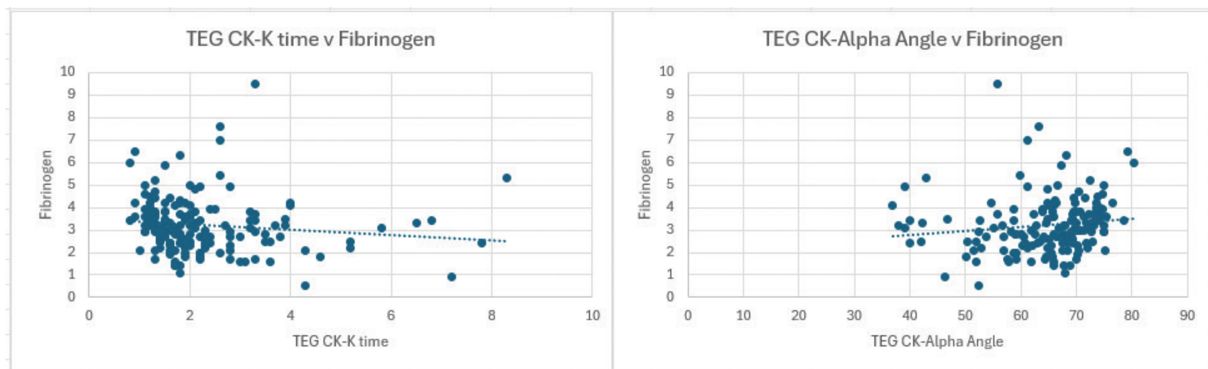
Table 1. *Correlation coefficients between TEG parameters and Laboratory tests. P value = derived from regression analysis. P value <0.05 are considered significant.*

TEG parameter	Laboratory test	R value	P value
R time	INR	0.0904	0.2513
R time	APTT	0.1423	0.0699
K time	Fibrinogen	-0.1188	0.1308
Alpha angle	Fibrinogen	0.1306	0.0965
MA	Fibrinogen	0.3933	<0.001
MA	Platelet Count	0.5775	<0.001

a. Clot initiation



b. Clot formation



c. Clot strength

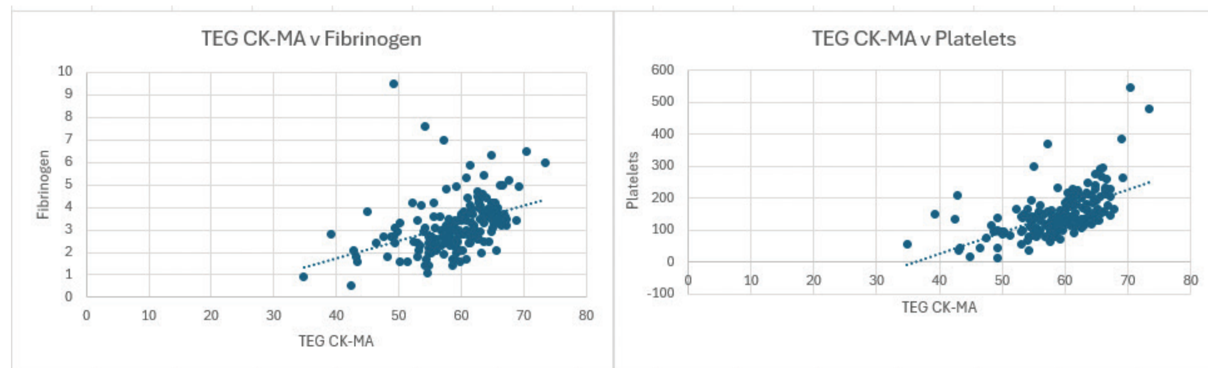


Figure 9a-c. *Correlations for clot initiation, formation and strength.*

According to the algorithm, if CFF-MA is less than 15mm and/or fibrinogen is less than 2g/L, cryoprecipitate should be transfused. CFF is the Citrated Functional Fibrinogen channel of the TEG® 6s cartridge, it provides clot strength base on fibrinogen contribution and is the most useful parameter to identify fibrinogen deficiency. The CFF assay contains kaolin and a glycoprotein IIb/IIIa antagonist providing MA based on fibrinogen contribution to the clot. The functional fibrinogen level is an estimation of plasma fibrinogen concentration as the platelet contribution to the clot is inhibited (Farkas 2021). It is appropriate to transfuse cryoprecipitate if fibrinogen is reduced as it is a plasma product consisting mostly of fibrinogen (Factor I), von Willebrand Factor and Factor VIII.

The CRT channel is the Rapid TEG test and as it has added tissue factor to enhance the coagulation process, it provides a quicker assessment of clot strength, without assessing clot initiation. In the Perioperative Algorithm, if the CRT-MA <50mm and/or the platelet count is less than 50 x 109/L then a platelet transfusion is indicated.

The last part of the algorithm examines the TEG CKH-R time parameter. The CKH channel is the Kaolin Heparinase Test and it is used to assess the effect of heparin. The heparinase in the test

neutralises any heparin in the patient's blood, thus making it the best test to identify clotting factor deficiency. If the CKH R time is greater than 10 minutes and/or the INR is greater than 1.5 then FFP is recommended for transfusion as it contains all coagulation factors including the labile plasma coagulation Factors VIII and V.

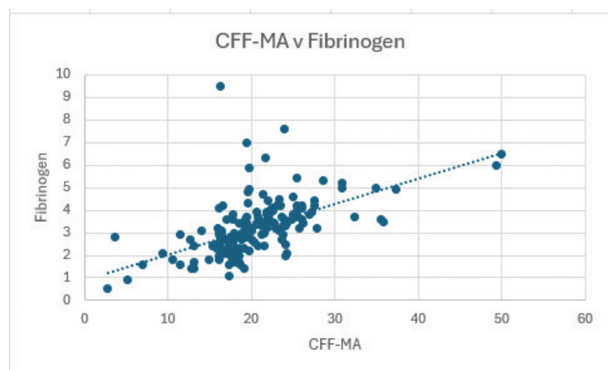
A comparison of the TEG CRT-MA to platelet count, TEG CKH-R and INR and CFF-MA and fibrinogen was conducted to identify how these relationships affect transfusion of blood products.

Data is presented as plots with linear fit (Figs 10 a-c), Pearson test r and P values (Table 2).

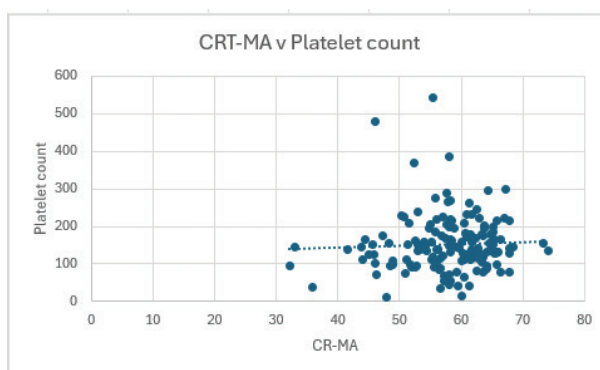
On examining the Perioperative Algorithm, the strongest correlations were between the Rapid TEG CRT-MA and platelet count (R=0.5848, P<0.001) and the functional fibrinogen TEG CFF-MA and fibrinogen level (R=0.5620, P<0.001). The weakest correlation was between TEG CKH-R times and INR (R=0.1902, P=0.0150).

To investigate if TEG parameters correlate to laboratory coagulation tests for how products were distributed, the data was examined in relation to products transfused and then sensitivity and specificity was calculated for each product. The sensitivity was defined as the ability of the TEG test to identify patients who need product based on the laboratory coagulation test, and specificity was defined as the ability of TEG test to not suggest product when the laboratory test was outside the algorithm range (Tables 3-5)

a.



b.



c.

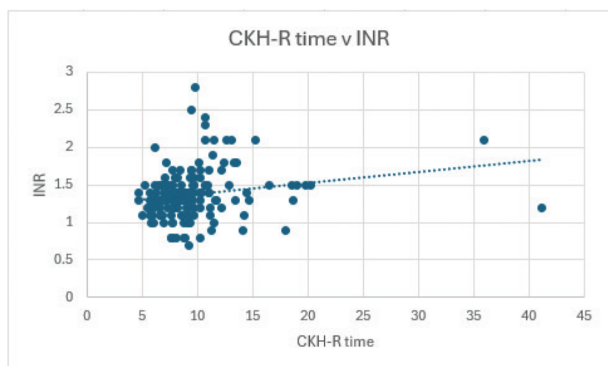


Figure 10a-c. *Correlations between TEG parameters and laboratory tests used in Monash Perioperative Algorithm*

Table 2. *Correlation coefficients between TEG parameters and laboratory tests used in Monash Perioperative Algorithm*

TEG parameter	Laboratory test	R value	P value
CFF MA	Fibrinogen	0.5620	<0.001
CRT MA	Platelet Count	0.5848	<0.001
CKH R	INR	0.1902	0.0150

Table 3. *The ability of a reduced TEG CRT-MA to detect a low platelet count: CRT-MA <50 and Platelet count <50*

CRTMA <50	Platelet <50 No	Yes	Total
No	134	9	143
Yes	15	5	20
Total	149	14	163

True D defined as platc_c ~= 0			[95% Conf.	Inter.]
Sensitivity	Pr(+| D)	35.71%	28.36%	43.07%
Specificity	Pr(-|~D)	89.93%	85.31%	94.55%
Positive predictive value	Pr(D| +)	25.00%	18.35%	31.65%
Negative predictive value	Pr(~D| -)	93.71%	89.98%	97.43%
Prevalence	Pr(D)	8.59%	4.29%	12.89%

Table 4. *The ability of an increased TEG CKH-R to detect an increased INR (CKH-R >10mm and INR>1.5)*

CKHR > 10	INR > 1.5 No	Yes	Total
No	85	29	114
Yes	23	26	49
Total	108	55	163

True D defined as inr_c ~= 0			[95% Conf.	Inter.]
Sensitivity	Pr(+| D)	47.27%	39.61%	54.94%
Specificity	Pr(-|~D)	78.70%	72.42%	84.99%
Positive predictive value	Pr(D| +)	53.06%	45.40%	60.72%
Negative predictive value	Pr(~D| -)	74.56%	67.88%	81.25%
Prevalence	Pr(D)	33.74%	26.48%	41.00%

Table 5. *The ability of a reduced TEG CFF-MA to detect a low fibrinogen (CFF-MA <15 and fibrinogen < 2 g/L)*

CCFMA < 15	Fibrinogen < 2 No	Yes	Total
No	139	9	148
Yes	6	9	15
Total	145	18	163

True D defined as fib_c ~= 0			[95% Conf.	Inter.]
Sensitivity	Pr(+| D)	50.00%	42.32%	57.68%
Specificity	Pr(-|~D)	95.86%	92.80%	98.92%
Positive predictive value	Pr(D| +)	60.00%	52.48%	67.52%
Negative predictive value	Pr(~D| -)	93.92%	90.25%	97.59%
Prevalence	Pr(D)	11.04%	6.23%	15.85%

Of the 14 people in the study with a low platelet (<50 x 109/L), a TEG CRT MA <50mm it was only detected this on 35% (5/14) occasions (sensitivity). If the platelet count was above 50 x 109/L (149 times), the TEG also agreed that it was high in 134/149 times or 89.93% (specificity).

Of the 55 people in the study with a high INR (>1.5), a TEG CKH R time >10 minutes (26/55) were detected giving a sensitivity of 47% . If the INR was below 1.5 (108 times), the TEG also agreed that it was low in 85/108 times or 78.70% (specificity).

Of the 18 people in the study with a low fibrinogen (<2.0), a TEG CFF MA time <15mm was detected on 50% (9/18) occasions (sensitivity). If the fibrinogen was above 2.0 (145 times), the TEG also agreed that it was low in 139/145 times or a specificity of 95.86%.

Even though the CRT-MA and platelet count had the strongest association, these parameters had the lowest rate of sensitivity for transfusion of platelets in the clinical setting. Of the 14 people in the study with a low platelet count (<50 x 109/L), a TEG CRT-MA <50mm only detected this on 35% of occurrences. The strongest predictor of product for transfusion was TEG CFF-MA and fibrinogen level with a sensitivity of 50% and specificity of 95.86% for the transfusion of cryoprecipitate.

Case study

To demonstrate how TEG and Laboratory tests are used to help direct transfusion during a bleeding event a case study is presented. The case was a 61-year-old female patient who attended VHH with inferior STEMI for an emergency coronary angiography. She went straight to the Catheter Laboratory and initial pictures showed possible ascending aortic dissection. The angioplasty was aborted for urgent surgical intervention. The patient went to the operating theatre for repair of the aortic dissection and CABG x2 and she was then admitted to ICU but returned to theatre due to haemorrhage and massive haemothorax. A MTP was activated and multiple products were issued. During this time she had seven TEG tests performed and six sets of laboratory blood tests including coagulation and FBEs. She was transfused 18 units of packed RBC, 13 units of FFP, 6 units of platelets and 45 units of cryoprecipitate. A graph of the 24 h during and after surgery, shows the TEG results, the laboratory coagulation results and the products transfused (Figs 11-13)

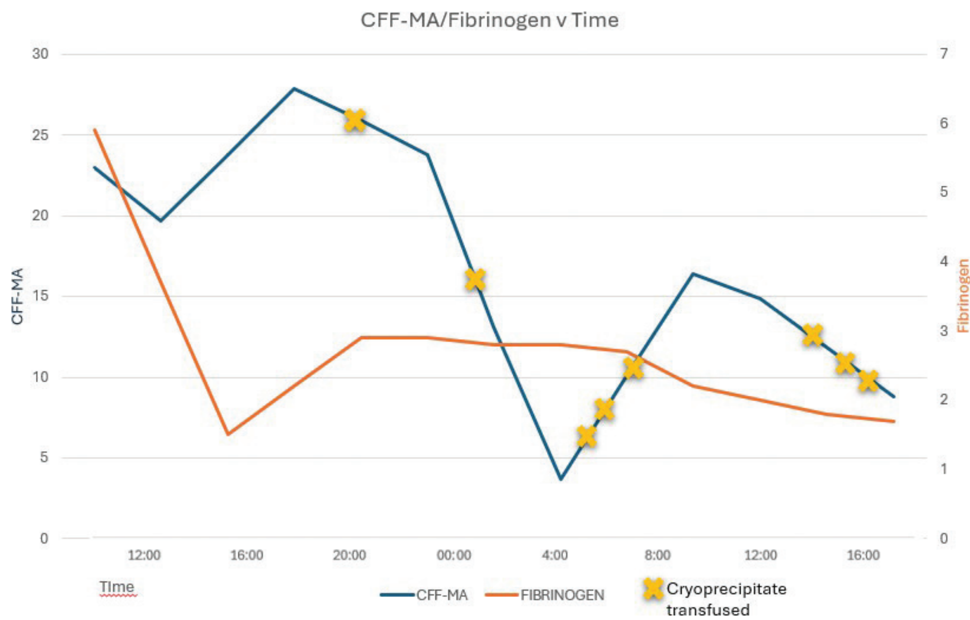


Figure 11. *TEG CFF-MA and fibrinogen levels over a 24 h period of time with cryoprecipitate transfusions noted.*

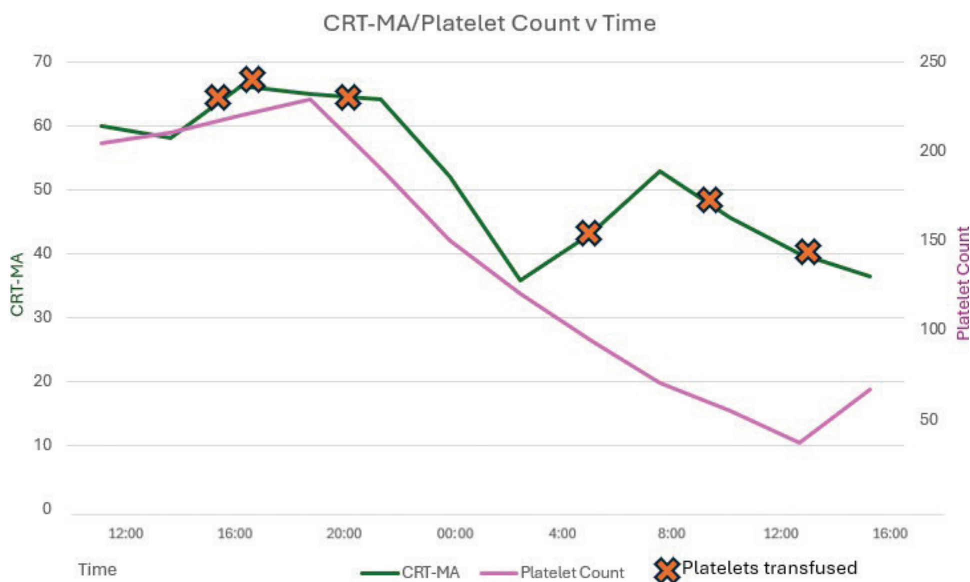


Figure 12. *TEG CRT-MA and platelet count over a 24 h period of time with platelet transfusions noted.*

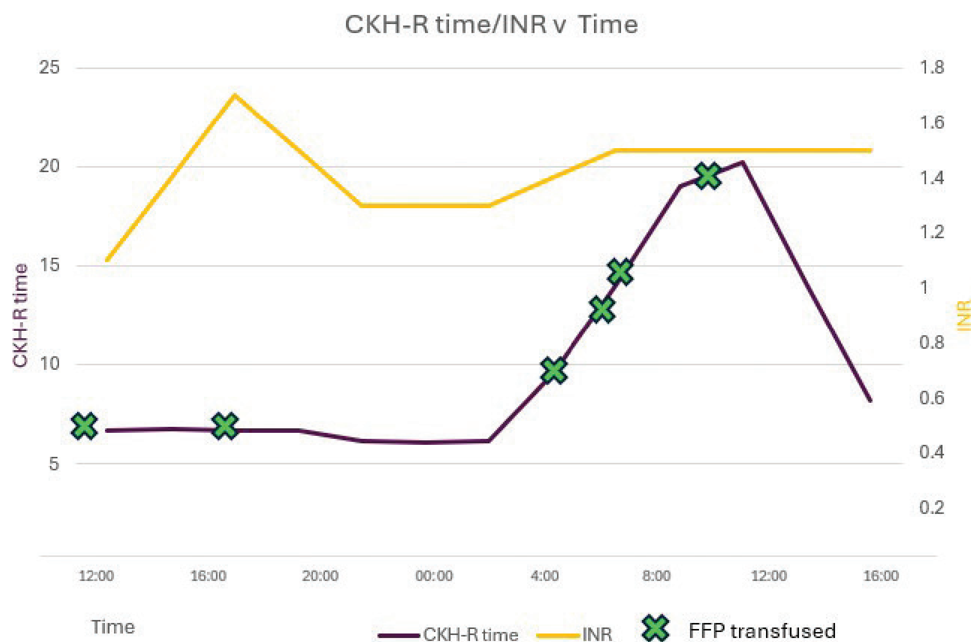


Figure 13. *TEG CKH-R time and INR over a 24 h period of time with FFP transfusions noted.*

Discussion

During the first year of VHH operating, 67 patients had MTPs activated and 46 patients required variations of Emergency Coagulation Packs. These episodes contributed to the transfusion of 586 units of packed RBC, 560 units of FFP, 283 units of platelets and 1281 units of cryoprecipitate. In these 113 patients, there were 16 patients that did not have a TEG test performed, and thus were excluded from analysis. The reasons for not performing a TEG may have been due to emergency nature of the bleeding event with a negative patient outcome, the TEG analyser may not have been available at the time or some clinicians may not subscribe to the idea of TEG analysis. Interestingly of the 16 patients without TEG results, all had laboratory coagulation results and these may have been relied upon for clinical decision making. There were also five patients that had multiple bleeding events that required activation of MTPs on different days. A retrospective analysis was done to compare the TEG results of these patients with their coagulation results. Initially all available TEG results were collated on the patient cohort, with some patients only having a single TEG analysis during their bleeding event whereas others had up to seven TEG tests performed. Not every TEG had laboratory coagulation tests drawn at the same time but most had coagulation tests within one hour. A total of 163 data sets of TEG and related INR/APTT/fibrinogen and platelet counts were analyzed.

There have been several studies comparing TEG parameters to laboratory coagulation tests in various patient populations. Results are quite inconsistent with studies showing varying relationships between the test platforms. The most common association is shown to be between TEG measures of clot strength and plasma fibrinogen level. This is important as deficiencies in fibrinogen have been associated with increased bleeding risk, so prompt treatment with cryoprecipitate can be vital for a positive outcome for patients with clinically significant bleeding. The weak correlations between TEG parameters and clotting initiation tests, INR and APTT have also been noted in the literature.

Since the TEG R times are the first parameters to be measured in the TEG system, with a normal range of up to 9.1 minutes and the threshold for transfusing being greater than 10 minutes, it supports the idea that early intervention with FFP may be more effective than waiting for traditional laboratory coagulation tests that can take up to 1 hour, even when processed urgently. Having a POC result within 10 minutes of blood drawn is more useful in decision making as it will better reflect the patient's coagulation state during surgery and would result in more timely delivery of blood products.

In general, the TATs for TEG are quicker than traditional laboratory tests. Typically, urgent laboratory tests have a maximum TAT of one hour. Once the blood samples are collected, they must be transported to the laboratory, checked for volume and correct labelling with patient details and then the tubes are received into the laboratory information system (LIS) and a unique laboratory identification barcode is printed. This is attached to the sample then centrifuged at 3800 rpm for 10 mins. There is only one centrifuge in specimen reception at VHH, so there are sometimes delays if it is already in use. Once centrifuged the coagulation samples are loaded onto the

ACLTOP 350 analyser and clotting profile is run which can take up to 15-20 mins. The results are downloaded to the LIS and they are reviewed by the scientist before being released. The results will only be available if the analyser has had all routine maintenance tasks completed and appropriate quality control has passed. In contrast, the Rapid TEG may be completed within 15 minutes with an average of 30-45 minutes processing time for a standard TEG. The quick TAT for a TEG result allows for assessment of the patient condition and earlier intervention with blood products if required.

The next part of this study was to compare the parameters used in the TEG-guided Perioperative Algorithm to assist decision making in the treatment of patients with clinically significant bleeding. The three key components of the Monash Algorithm were examined to determine their relationships and how the results related to the transfusion of cryoprecipitate, platelets and FFP. It has been noted that there are many different transfusion algorithms currently in use across different sites, using various combinations of TEG parameters and thresholds to guide transfusion. There is not one recommended algorithm, however generally any TEG guided algorithm seems to decrease product usage, even if different parameters are being used. More data needs to be collected to better understand the use of TEG in the cardiac surgical setting if we are to move towards a more universally accepted algorithm. The reason for so many variations may be due to the different patient populations, however even within the cardiac setting it was surprising to see so many different versions of the transfusion algorithm.

The final analysis involved investigation of blood products transfused during and/or after surgery and how well the algorithm predicts products used in relation to TEG and/ or laboratory coagulation tests. Even though the CRT-MA and platelet count had the strongest association, these parameters had the lowest rate of sensitivity for transfusion of platelets in the clinical setting (35%). The strongest predictor of product transfusion was TEG CFF-MA and fibrinogen level with a sensitivity of 50% and specificity of 95.86% for the transfusion of cryoprecipitate. The case study showed that during a 24 h period a bleeding episode can have a varied and unpredictable pathway. At several points throughout the event. TEG and laboratory tests were used to aid in decision making for blood products for transfusion. The results did not always correlate well with regard to the Perioperative Algorithm, however this may have been due time differences in collection of the TEG and laboratory tests samples and/or rapidly evolving bleeding event and surgical intervention.

From a laboratory perspective, it has been interesting to learn about the differences in Quality Control (QC) and Quality Assurance (QA) when it comes to TEG and laboratory coagulation tests. The accuracy of laboratory results is of highest priority with each test platform having strenuous and thorough quality procedures in place to deliver accurate results. The ACL TOP 350, has multiple quality control samples run at a four hourly schedule to ensure consistency and accuracy of INR,

APTT and fibrinogen tests. The DxH 690T also has multiple quality control samples of different levels run on a four hourly basis. QC is reviewed daily, weekly and monthly to identify trends and any issues to do with instrumentation, reagents and testing procedures. A weekly interlaboratory quality control sample is also conducted across the different Monash sites to ensure consistency and accuracy. An external QA program (RCPA EQAP) is used to compare data to other users of instrumentation and reagents as a further measure of test accuracy. All of these quality systems are in place and monitored under NATA accreditation guidelines to ensure that the laboratory results are valid and reliable. On the other hand, TEG QC measures are determined by hospital policy and configured by the administrator. Different institutions may therefore run different QC schedules. At Monash two levels of QC material should be run once a week, however it is possible to run the analyser after a failed QC result. The TEG analysers are also not part of the laboratory service, so responsibility of quality control review and action falls to theatre/ICU staff who may or may not be engaged and/or trained to handle QC appropriately. The analysers are also enrolled in an external QA program that is run twice a year. The differences in TEG and laboratory testing QC regimes do suggest that there may be more risk associated with the POC system as it is not monitored as closely and this may affect accuracy of the TEG results.

It has also been interesting to examine TEG and its use from a laboratory perspective as the laboratory staff have limited knowledge of this POC system. At Monash the pathology laboratory processes the coagulation and FBE samples as well as issuing the blood products from the Blood Bank. In the data studied there were 117 episodes of bleeding events at VHH with 42% of these occurring out of hours when there is only one scientist working in the laboratory, so the same scientist performing the laboratory tests is the one issuing products from the blood bank. The laboratory staff at VHH are not trained in the use of TEG and do not have access to the TEG results, so the request for blood products may be directed by TEG results but this is a missing link for the pathology staff. The laboratory will always deliver timely blood test results and provide blood products as required but it is also helpful to have knowledge of other methods of decision making to better understand and anticipate the situation when dealing with a bleeding patient.

The study has several limitations including size of patient

cohort and more data may be useful to further identify relationships between the TEG parameters and laboratory coagulation tests. Also, as this was a retrospective study, not all TEG tests had corresponding coagulation samples collected and sent for analysis. Sometimes the next available set of laboratory results were included and the difference in collection time could make a difference to the correlations as the patient may have had a change in coagulation state, especially if blood products has been transfused during this time.

Another limitation was that anticoagulants and anti-platelet agents were not taken into consideration or was the infusion of protamine or transexamic acid. These factors can all affect patient haemostasis, as well as the clinical situation, so interpretation of any coagulation test results must be done with caution.

Conclusion

In cardiac patients at VHH it has been shown there are varying relationships between TEG parameters and laboratory coagulation tests. The strongest associations occurred when measuring clot strength. TEG parameter CK-MA and laboratory tests for fibrinogen and platelet count appeared to correlate well compared to other parameters. The weakest correlations with the normal TEG tests were between CK-R time and INR, which is interesting as these tests are closest to those appearing on the Perioperative Algorithm for recommendation of FFP transfusion.

The comparisons for the algorithm tests showed better correlation to those from the normal TEG results. The TEG parameters of interest included three from the other channels of the TEG® 6s cartridge; CFF-MA, CRT-MA and CKH-R. When these were compared to laboratory tests of platelet count, fibrinogen level and INR, the strongest association was between CRT-MA and platelet count, followed closely with CFF-MA and fibrinogen. The weakest association was between CKH-R and INR.

Both POC and laboratory results, together with cardiac surgeon's clinical experience and clinical setting, contribute to decision making for transfusion of blood products in patients with significant bleeding. TEG appears to provide the preferred parameters to guide transfusion due to the quick TAT and reliable results, however not all clinicians utilize TEG in their decision making. The TEG MA measurements have the strongest agreement with laboratory platelet counts and fibrinogen levels however the transfusion of cryoprecipitate is more likely than platelet transfusion when following the Perioperative Algorithm for transfusion. Laboratory tests are also useful in guiding patient care, however the long TAT can mean the TEG is the preferred method of evaluating patient haemostasis during and post-surgery. It may be helpful for the laboratory to have a better understanding of TEG and it's use in monitoring bleeding episodes. This would allow for scientists to be better prepared for blood product requirements during MTPs, particularly if the laboratory staff are working alone.

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ORIGINAL ARTICLE

Virtual Microscopy Imager (VMI): a digital pathology solution for undergraduate Laboratory Medicine students

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Abstract

Whole slide imaging and virtual microscopy enable the digitisation and analysis of pathology specimens on glass microscopy slides for diagnostic, quality assurance and educational purposes. To prepare Laboratory Medicine graduates for virtual microscopy, we have developed Virtual Microscopy Imager (VMI), a cost-effective virtual microscopy platform. This study sought quantitative and qualitative feedback from Laboratory Medicine students on Version 1.0 of VMI via an online survey. Results demonstrated that students consider VMI useful for study and easy to use on a variety of devices both on and off campus. The differences between second- and fourth-year students in their preferred study tools are also highlighted. Additional functionality suggested by students, including annotations, an information panel, a measurement tool, and a leucocyte differential counter, was integrated into VMI Version 2.0. VMI represents a robust virtual microscopy platform that enables students to study histopathology and cytology curriculum in a flexible manner.

*Keywords: **Digital pathology, higher education, diagnostic pathology, virtual microscopy, Laboratory Medicine, whole slide imaging, histopathology, cytology***

Introduction

Histopathology and cytology involve microscopic evaluation to assist with patient diagnosis and clinical management. Light microscopy has been taught since the end of the 19th century (Gu and Ogilvie 2005) and Laboratory Medicine students at our Institution have traditionally used this technique to examine cellular smears or tissue sections. More recently light microscopy has been digitally emulated using whole slide imaging (WSI) and virtual microscopy (VM). In WSI, a glass microscopy slide is scanned to produce a high-quality digital image prior to VM analysis (Gupta *et al* 2022). Digitisation of slides for pathological examination was first reported in 1987 (Weinstein *et al* 1987). VM has subsequently grown exponentially with most Histopathology laboratories now working towards a fully digitised diagnostic laboratory.

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Laboratory Medicine students need to develop the skills required to appreciate the differences between light and VM in the diagnostic context as this will enhance their skills ready for the workplace. In medical education VM has the additional benefits of the concurrent presentation of identical specimens to learners, the inclusion of digital annotations to aid learning, and specimen preservation, including those from rare cases (Kuo and Leo 2019).

In 2008, academics at our Institution used an Aperio Scanscope CS to perform WSI of approximately 50 histopathology slides and purchased Aperio Spectrum, which allowed students to remotely analyse whole slide images (WSIs). The server running this solution was deprecated in 2018 and its replacement (Aperio eSlide Manager) was cost prohibitive for our Institution. As an alternative, students were asked to download Aperio ImageScope and individual WSIs for analysis. Students reported that the images were difficult to navigate and the large WSIs file size (up to 15-40GB per image, depending on the objective at which the slide is scanned) (Park *et al* 2012) prohibited their download by those with limited Internet access. Students became despondent and disengaged with viewing the material and clearly, an alternative was required.

Other commercially available solutions, including Pathozoom, SmartZoom ClassRoom (MyObjective Cloud, Sectra and Cytomine were investigated but were found to be cost prohibitive for our Institution. Free slide repositories, such Dr Lee's Histology Laboratory, the AMBOSS Virtual Histopathology Slide Box, the National University of Singapore School of Medicine's Normal Histology, the University of Leeds' Virtual Pathology Slide Library and the Virtual Microscopy Database (Lee *et al* 2018)) are available but do not correlate well with our course curriculum.

We therefore built VMI, a custom VM platform that is compatible with our Institution's learning management system (LMS) and the previously mentioned WSIs. This project used an online Qualtrics survey (Appendix 1) to seek the student voice on VMI, specifically to learn how they were using it in their study of histopathology and cytology, and their opinion on potential VMI improvements to help guide its development.

Materials and methods

Ethics

Approval to complete this study was obtained from the Curtin University Human Research Ethics Committee (approval number HREC2019-0294).

Slide scanning

A Zeiss Axioscan 7 (Carl Zeiss Pty Ltd, NSW, Australia) was used to scan slides. This scanner is housed in the Curtin Health Innovation Research Institute (CHIRI) situated on Curtin's Bentley Campus. It is freely available for use by CHIRI investigators. It generates files in the Carl Zeiss Image (CZI) format. CZI files were opened in ZEN (Blue Edition, version 3.4; Carl Zeiss Microscopy GmbH) and saved as TIFF files. WSIs generated during 2008 have the Tiled TIFF (SVS) format. At the time of this study, our WSI repository contained approximately 140 slides in total (50 SVS, 90 CZI). All were available to students for viewing.

Stained slides prepared from histopathology tissue sections and Hologic ThinPrep liquid-based cytology (LBC) samples were scanned using a 20X objective lens. The range of histopathology cases included normal adrenal, aorta, bladder, bone, kidney, large bowel, liver, lung, lymph node, ovary, pituitary, salivary gland, skeletal muscle, cerebellum, and cerebrum, and pathological entities demonstrating classical features including breast ductal carcinoma, breast fibroadenoma, cardiac hypertrophy, gastric antrum containing *Helicobacter pylori*, liver cirrhosis, *Cryptococcus* sp. in lung, lung metastatic melanoma, and a tuberculosis (TB) granulomatous lesion in lung. Stained smeared serous effusion, cerebrospinal fluid, sputum and urine samples, and a range of fine needle aspirate (FNA) cytology samples, including breast, lymph node, salivary gland and thyroid, were scanned using a 40X objective lens to enable viewing of cellular crowding and overlapped cellular material.

Virtual microscopy imager (VMI)

Version 1.0 of VMI (Figure 1) was developed in the first half of 2019 using the open-source Open Seadragon software and deployed onto our LMS (Blackboard; Blackboard Inc., now part of Anthology, Reston, VA, USA) as a Sharable Content Object Reference Model (SCORM) package. It is available only to Curtin University students. Before viewing SVS or TIFF files in VMI, they must be converted to the opensource Deep Zoom Image (DZI) format. The DZI format separates the whole slide image into a series of tiles and uses a 'tiled pyramid' approach to mimic the magnification of microscope objective lenses. McClintock *et al* (2022) have detailed this process (McClintock *et al* 2022). SVS/TIFF to DZI file conversion was achieved using Tifffile VIPand custom Python scripts. DZI files were stored in an S3 bucket in Curtin University's Amazon Web Services (AWS) environment to be accessed directly by VMI.

Study design

Two student cohorts were surveyed in this study. PATH2001 (*Foundations of Pathology*) is a second- year 14-week full semester introductory pathology unit completed by all Laboratory Medicine students at Curtin University. Content is delivered via weekly face-to-face lectures, tutorials and laboratory classes. During each laboratory session, students were directed to use VMI on their personal electronic device(s) (laptop, tablet, and/or smartphone) to view WSIs that correlated to specimens prepared in class and analysed by light microscopy. This allowed them to check whether they had correctly performed the required staining procedure. Students also have access to laboratory facilities outside of their scheduled class hours (but only during business hours) that house light microscopes, allowing them to review microscopy slides prepared during laboratory classes. Students are also provided with online recorded video tutorials, delivered via the University's LMS, that summarise the histopathological findings in the cases investigated.

MEDI4001 (*Advanced Diagnostic Cytology*) was a fourth-year 14-week full semester unit available as part of the Cytology major within the Laboratory Medicine course but content from this unit is now taught in MEDI4010 (*Advanced Anatomical Pathology*). It was taken by students after completion of their clinical placement. The content was delivered via weekly face-to-face lectures, tutorials and laboratory classes. These students have access to a

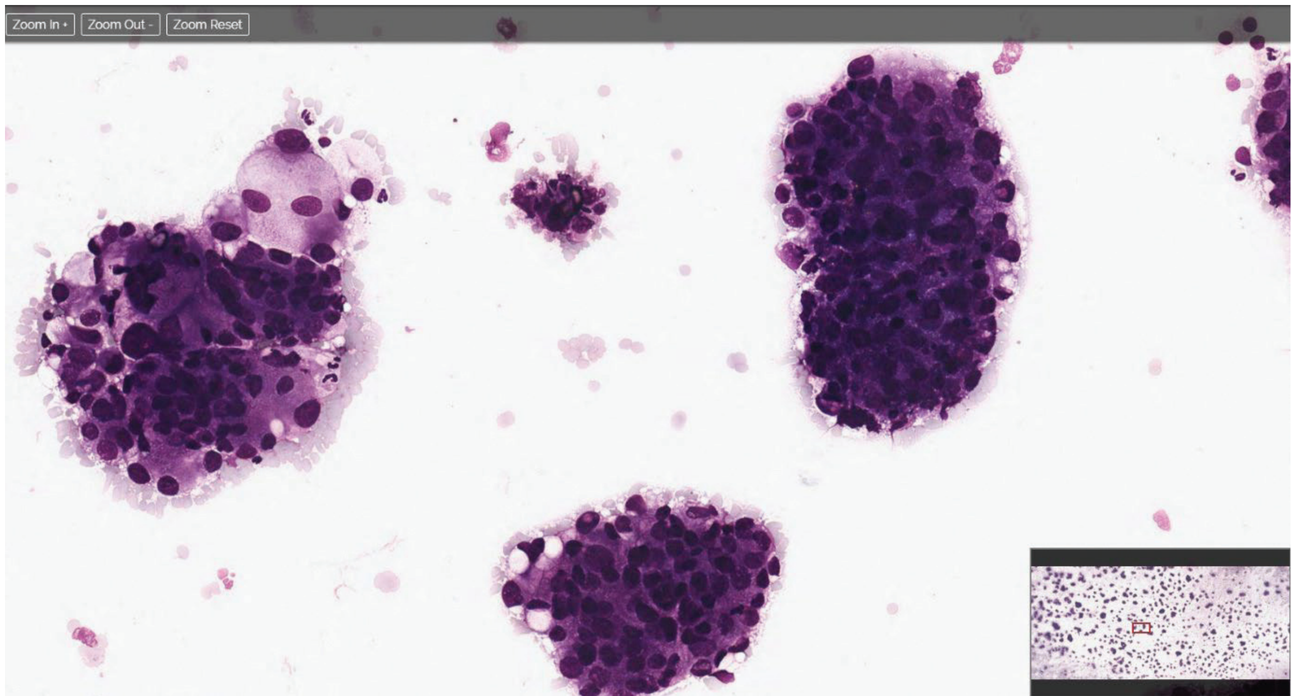

Figure 1. *Example of VMI, version 1.0. This version contained basic functionality, only allowing students to navigate the slide and zoom in/out. VMI allows the user to magnify the image to an equivalent of the lens used to scan the slide i.e. 20x or 40x; it does not permit digital magnification. The specimen shown is a cytopathology smear of an ovarian cyst fluid stained with Diff Quik.*

dedicated microscopy room for unsupervised use. They review weekly cases presented during the laboratory sessions and an additional 5-7 cases per week that they are expected to analyse outside of class. Due to limited sample availability, some of these additional cases are available only in VMI. MEDI4001 students were informed that VMI was a supplementary learning resource available for use outside of class to advance their diagnostic skills.

In semester 2 2019, VMI (version 1.0) was made available to both cohorts (PATH2001 n=67 and MEDI4001 n=18). In the first practical class, the laboratory demonstrator introduced students to VMI and instructed them on how to access it within the University's LMS. Their access remained for the duration of semester allowing them to use VMI to learn content in class or as a revision tool when off campus. In week 8 of the teaching semester, a voluntary online Qualtrics survey (Provo, UT, USA) was made available for students to provide quantitative and qualitative feedback on VMI. This survey was accessible until the end of semester (i.e. until week 14). The full survey is provided Appendix 1 and 2. Question 21 - 'How would you rate the following functionalities of the imager?' required participants to provide their response on a five-point Likert scale: Extremely Poor (1); Poor (2); Neutral (3); Good (4); Extremely Good (5). This data was analysed by converting text responses to their numerical equivalent (shown in brackets in the preceding sentence) and the median response determined. Question 22 required participants to rank potential VMI improvements in order of importance for their study/revision purposes, and questions 25 and 26 required participants to rank several study tools in order of frequency of use and perceived benefit. Responses from these questions are presented as the mean rank ± SD, as provided in the default Qualtrics survey report. Data from remaining quantitative questions is presented as the number of responses and the percentage of total responses. For analysis of qualitative data, themes were extracted from all responses and the number of responses related to each theme were collated. Complete quantitative responses are provided in. Appendix 2.

Results

All students enrolled in PATH2001 and MEDI4001 (n=85) were invited to participate in this study with 30 (35%) consenting to do so. This response rate is similarto that in other VM studies (Caruso 2021; Samueli *et al* 2020). Basic demographic data of the survey respondents is presented in Table 1. Most participants were aged 18-25 (83.3%) and identified as female (86.7%). These data are consistent with a study performed in a large first-year human biology unit (HUMB1000) that is completed by all Laboratory Medicine students at our Institution (Fyfe *et al* 2018). Most respondents (80%) were enrolled in PATH2001 with the remainder (20%) in MEDI4001, which is representative of the overall student distribution between units (PATH2001 78.8%; MEDI4001 21.2%).

We initially investigated the student use of VMI (Table 2). Most respondents (90%) reviewed 6-30 images during the study period. Most PATH2001 respondents (75%) viewed 11-30 images. Conversely only 33% of those enrolled in MEDI4001 viewed a similar number and the remaining 67% only viewed 6-10. Most PATH2001 students used VMI for 10-30 or 30-60 mins/week (29.2% and 33.3%, respectively). In contrast, most MEDI4001 students (50%) used it for 10-30 mins/week. Most students (86.6% of respondents) found VMI either very or extremely useful for study/revision.

Table 1. *Demographics of survey respondents.*

Age	n (%)
18-20	12 (40.0%)
21-25	13 (43.3%)
26-30	3 (10.0%)
31-40	1 (3.3%)
40+	1 (3.3%)
Gender	
Male	4 (13.3%)
Female	26 (86.7%)
Student type	
Domestic	27 (90.0%)
International	3 (10.0%)
Unit of study	
PATH2001	24 (80.0%)
MEDI4001	6 (20.0%)

Table 2. *VMI 1.0 use during the study period.*

	PATH2001 (n=24)	MEDI4001 (n=6)	Total (n=30)
Number of images viewed in VMI			
1-5	1 (4.2%)	0 (0.0%)	1 (3.3%)
6-10	4 (16.7%)	4 (66.7%)	8 (26.7%)
11-20	8 (33.3%)	1 (16.7%)	9 (30.0%)
21-30	10 (41.7%)	1 (16.7%)	11 (36.7%)
31-40	1 (4.2%)	0 (0.0%)	1 (3.3%)
41-50	0 (0.0%)	0 (0.0%)	0 (0.0%)
Time spent per week using VMI			
1-5	1 (4.2%)	0 (0.0%)	1 (3.3%)
6-10	4 (16.7%)	4 (66.7%)	8 (26.7%)
11-20	8 (33.3%)	1 (16.7%)	9 (30.0%)
21-30	10 (41.7%)	1 (16.7%)	11 (36.7%)
31-40	1 (4.2%)	0 (0.0%)	1 (3.3%)
41-50	0 (0.0%)	0 (0.0%)	0 (0.0%)
Usefulness of VMI for study/revision			
Extremely useful	11 (45.8%)	2 (33.3%)	13 (43.3%)
Very useful	11 (45.8%)	2 (33.3%)	13 (43.3%)
Moderately useful	1 (4.2%)	1 (16.7%)	2 (6.7%)
Slightly useful	1 (4.2%)	1 (16.7%)	2 (6.7%)
Not useful at all	0 (0.0%)	0 (0.0%)	0 (0.0%)

We were next interested to learn location and technology-related aspects of VMI use (Table 3). Just over half of respondents (56.7%) used VMI on campus. All used it on their laptops and the majority (88.2%) were enrolled in PATH2001. Six students also used it on their tablet and/or smartphone while on campus. All students used VMI off campus. Most used it on a laptop (90%) while 46.7% also used it on their tablet and/or smartphone connected via WiFi or mobile network. Irrespective of the location or network type used to access VMI, most respondents perceived the speed as 'acceptable' or 'fast'. VMI was most used in Google Chrome, followed by Apple Safari and Microsoft Edge, consistent with the browser market share as of September 2023 (https://gs.statcounter.com/browser-market-share/desktop/australia).

Table 3. *VMI 1.0 use during the study period.*

	PATH2001 (n=24)	MEDI4001 (n=6)	Total (n=30)
Location where VMI was used			
On campus	15 (62.5%)	2 (33.3%)	17 (56.7%)
Off campus	24 (100.0%)	6 (100.0%)	30 (100.0%)
On campus use			
Devices used to access VMI			
University computer	2 (13.3%)	1 (50.0%)	3 (20.0%)
Laptop	15 (100.0%)	2 (100.0%)	17 (100.0%)
Tablet	3 (20.0%)	0 (0.0%)	3 (20.0%)
Smartphone	3 (20.0%)	0 (0.0%)	3 (20.0%)
Perceived speed of image-loading			
Extremely slowly	0 (0.0%)	0 (0.0%)	0 (0.0%)
Slowly	2 (13.3%)	0 (0.0%)	2 (11.8%)
Acceptable	7 (46.7%)	0 (0.0%)	7 (41.2%)
Fast	4 (26.7%)	2 (100.0%)	6 (35.3%)
Extremely fast	1 (6.7%)	0 (0.0%)	1 (5.9%)
Instantly	1 (6.7%)	0 (0.0%)	1 (5.9%)
Off campus use			
Devices used to access VMI			
Desktop computer	4 (16.7%)	2 (33.3%)	6 (20.0%)
Laptop	21 (87.5%)	6 (100.0%)	27 (90.0%)
Tablet	5 (20.8%)	0 (0.0%)	5 (16.7%)
Smartphone (WiFi)	4 (16.7%)	0 (0.0%)	4 (13.3%)
Smartphone (Mobile)	4 (16.7%)	1 (16.7%)	5 (16.7%)
Perceived speed of image-loading over WiFi			
Extremely slowly	0 (0.0%)	0 (0.0%)	0 (0.0%)
Slowly	2 (8.4%)	0 (0.0%)	2 (6.7%)
Acceptable	9 (37.5%)	3 (50.0%)	12 (40.0%)
Fast	9 (37.5%)	3 (50.0%)	12 (40.0%)
Extremely fast	4 (16.7%)	0 (0.0%)	4 (13.3%)
Instantly	0 (0.0%)	0 (0.0%)	0 (0.0%)
Perceived speed of image-loading over mobile network			
Extremely slowly	0 (0.0%)	0 (0.0%)	0 (0.0%)
Slowly	0 (0.0%)	0 (0.0%)	0 (0.0%)
Acceptable	2 (8.4%)	0 (0.0%)	2 (6.7%)
Fast	1 (4.2%)	1 (16.7%)	2 (6.7%)
Extremely fast	1 (4.2%)	0 (0.0%)	1 (3.3%)
Instantly	0 (0.0%)	0 (0.0%)	0 (0.0%)
Browser used to access VMI			
Google Chrome	19 (79.2%)	5 (83.3%)	24 (80.0%)
Mozilla Firefox	1 (4.2%)	1 (16.7%)	2 (6.7%)
Microsoft Edge	5 (20.8%)	1 (16.7%)	6 (20.0%)
Microsoft Internet Explorer	4 (16.7%)	0 (0.0%)	4 (13.3%)
Apple Safari	8 (33.3%)	1 (16.7%)	9 (30.0%)

Table 4. *Ratings of VMI 1.0 functionality. Respondents were asked to rank the zoom and navigation functions, and image resolution, on a five-point Likert Scale. EP (1) = Extremely Poor; P (2) = Poor; N (3) = Neutral; G (4) = Good; EG (5) = Extremely Good. The median (Mdn) response was determined using the corresponding numerical values shown in brackets.*

Image	**PATH2001 (n=23)**						**MEDI4001 (n=6)**					
	EP (1)	P (2)	N (3)	G (4)	EG (5)	Mdn	EP (1)	P (2)	N (3)	G (4)	EG (5)	Mdn
Zooming	0 (0.0%)	1 (4.3%)	1 (4.3%)	13 (56.5%)	8 (34.8%)	4	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (66.7%)	2 (33.3%)	4
Navigation	0 (0.0%)	1 (4.3%)	1 (4.3%)	13 (56.5%)	8 (34.8%)	4	0 (0.0%)	0 (0.0%)	1 (16.7%)	4 (66.7%)	1 (16.7%)	4
Resolution	0 (0.0%)	0 (0.0%)	2 (8.6%)	10 (43.5%)	11 (47.8%)	4	0 (0.0%)	0 (0.0%)	2 (33.3%)	2 (33.3%)	2 (33.3%)	4

Table 5. *Tools used for study/revision purposes. Rank values (Mean ± SD) were obtained from the Default Report generated in Qualtrics. The highest rank values for each unit are italicised.*

	PATH2001 (n=23)	**MEDI4001 (n=6)**
Tools used for study/revision (n, %)		
Light microscopy	18 (78.3%)	6 (100.0%)
The digital slide imager used in this study (VMI 1.0)	23 (100.0%)	6 (100.0%)
Static images, from either lecture notes, a book, or online	22 (95.7%)	6 (100.0%)
Another digital slide imager/catalogue available online	3 (13.0%)	3 (50.0%)
Video tutorial provided by lecturer/demonstrator/tutor in your unit	21 (91.3%)	4 (66.7%)
YouTube/other online video	8 (34.8%)	3 (50.0%)
Rank by frequency of use (Mean ± SD)		
Light microscopy	2.91 ± 1.18	1.50 ± 0.50
The digital slide imager used in this study (VMI 1.0)	2.43 ± 0.97	3.67 ± 1.25
Static images, from either lecture notes, a book, or online	2.57 ± 1.31	3.00 ± 0.58
Another digital slide imager/catalogue available online	5.61 ± 0.49	5.33 ± 0.47
Tutorial provided by lecturer/demonstrator/tutor in your unit	2.30 ± 1.04	2.17 ± 1.34
YouTube/other online video	5.17 ± 1.05	5.33 ± 0.75
Rank by benefit (Mean ± SD)		
Light microscopy	3.24 ± 1.11	1.50 ± 0.50
The digital slide imager used in this study (VMI 1.0)	2.43 ± 0.95	3.25 ± 0.83
Static images, from either lecture notes, a book, or online	3.24 ± 1.34	3.25 ± 0.43
Another digital slide imager/catalogue available online	5.62 ± 0.58	5.25 ± 0.43
Tutorial provided by lecturer/demonstrator/tutor in your unit	1.81 ± 1.01	2.00 ± 1.22
YouTube/other online video	4.67 ± 1.49	5.75 ± 0.43

Table 6. Rank of requested features for version 2.0 of VMI. Rank values (Mean ± SD) were obtained from the Default Report generated in Qualtrics. The highest rank values for each unit are italicised.

Requested feature	PATH2001 (Mean ± SD; n=21)	MEDI4001 (Mean ± SD; n=6)
The ability to see annotations from your lecturer/tutor/demonstrator	1.67 ± 1.04	2.33 ± 1.37
The ability to annotate images yourself	2.48 ± 0.96	2.33 ± 0.47
The ability to highlight particular cells or regions of the slide	2.71 ± 1.12	2.17 ± 1.34
An indication of the slide area that you have viewed already	3.95 ± 1.33	3.83 ± 1.07
The ability to see annotations from your classmates	4.62 ± 1.21	4.33 ± 0.94
Inclusion of a digital counter for cell counting purposes	5.57 ± 0.49	6.00 ± 0.00

Participants were next asked about the functionality of VMI (Table 4). When asked about the image resolution, or the ability to zoom or navigate the image, the median response was 'Good'.

We were interested to learn how students perceived VMI compared to other tools for study/revision purposes (Table 5). Respondents were first asked which of several modalities they used for this purpose. All used VMI and all but one (96.6%) used static images obtained from a lecture, book, or online. All MEDI4001 students used light microscopy, as did a lesser but significant proportion (78.3%) of PATH2001 students. Most respondents enrolled in PATH2001 (91.3%) used a tutorial provided by an academic (lecturer/demonstrator/tutor) with a lesser number in MEDI4001 (66.7%) using the same tool.

Respondents were then asked to rank each of the tools by frequency of use for study/revision purposes and their perceived benefit to their learning. PATH2001 respondents indicated that a tutorial provided by an academic was most frequently used and was most beneficial to their learning, followed by VMI in both categories. Conversely, MEDI4001 respondents indicated that light microscopy was most frequently used and was most beneficial to their learning. A tutorial provided by an academic followed in both categories. For this cohort, VMI ranked fourth in both categories.

We sought respondent feedback on potential VMI improvements in the final questions. Students were asked to rank the following features by order of importance for study/revision purposes; annotations from academics, annotations from peers, self-annotation, the ability to highlight cells/regions of interest, an indication of the slide area viewed, or inclusion of a cell counter (Table 6). PATH2001 respondents ranked annotations from academics as most important, followed by self-annotation and the ability to highlight cells/regions of interest. MEDI4001 respondents ranked the ability to highlight cells/regions of interest as most important, closely followed by self-annotation and annotations from academics. Respondents were also afforded the opportunity to provide additional suggestions via a free-text box. Most responses (9) related to the inclusion of sample details (i.e. tissue type, disease state, stain used). Additional responses (1 of each) suggested inclusion of a discussion board, guided specimen 'tours', and formative assessments.

Discussion

VMI, a virtual microscopy platform to accommodate the histopathology and cytology curriculum in the Laboratory Medicine course was developed at Curtin University. Data from this study indicates that students found version 1.0 of VMI useful for viewing WSIs on any device, on or off campus, and at an acceptable or better speed. They found it very useful for their study as it allowed them to revise learning material at a time and location of their choosing without a conventional microscope.

The two surveyed cohorts displayed notable differences in several areas of this study. PATH2001 students viewed more images in VMI and spent more time per week using it. Given that these students have little pathology and microscopy experience compared to MEDI4001 students, it is reasonable to expect that they would use a supplementary learning resource more to aid their learning. PATH2001 students also exhibited more on-campus VMI use. This is also not surprising, given that they were directed to use VMI during laboratory classes to check their sections for correct staining against those in VMI. In contrast, MEDI4001 students were informed that VMI was a supplementary learning resource for use outside of class.

There were also differences in the various study/revision tools that the two cohorts used. PATH2001 respondents used pre-recorded videos most frequently for study/ revision purposes and found them most beneficial for their learning, followed by VMI in both categories. There are two possible explanations for this. PATH2001 is an introductory pathology unit for second-year Laboratory Medicine students with limited pathology knowledge., A didactic learning environment for understanding the fundamentals of various pathologies may therefore be more beneficial for these students (Wong 2022). Secondly these students used version 1.0 of VMI which did not contain the annotation functionality. Their viewing of WSIs was therefore akin to traditional light microscopy in that the image could only be viewed, navigated, and magnified. The annotation functionality included in version 2.0 of VMI allows academics to provide an infinite amount of detailed information on cells, structures, and regions of interest on WSIs, facilitating incorporation of the equivalent information that was included in pre-recorded videos within a student-centred, active learning environment. These environments can increase a students' motivation to learn and lead to enhanced understanding and retention of knowledge (O'Brien and Collins 2003). Indeed, annotation functionality in VMs has been demonstrated to improve engagement with course content, promote group discussion, and increase junior learner performance (Harris *et al* 2001; Maybury and Farah 2009; Bloodgood 2012; Sahota *et al* 2016; Rinaldi *et al* 2017).

A large percentage (≈80%) of PATH2001 students used light microscopy for study/revision but all MEDI4001 students used this tool for the same purpose. Of all the study/revision tools available, the latter cohort used light microscopy most frequently and found it most beneficial to their learning. Notably they ranked VMI as fourth in these areas. This aligns with their significant light microscopy experience, the requirement for them to review specimens on glass slides outside of class, and the availability of a dedicated microscopy suite where they could learn within a community of practice. One student noted that it was difficult to observe the cellular details in cytological samples in VMI due to their three-dimensional nature - *"I found challenges in being able to access cellular details in approximately half of the area for most images due to the image being out focus in those areas. …It made it difficult to help assess cells for abnormalities."*. A qualified cytologist with 20+ years of diagnostic laboratory experience reviewed our cytology WSIs and indicated that there was no evidence of imaging artifact in them. the MEDI4001 cohort might therefore have struggled with the analysis of cytology WSIs due to their relative lack of experience in viewing them, as has been described in other studies (Wilbur 2011; Evered and Dudding 2011; Donnelly *et al* 2013; Rajaganesan *et al* 2021). It is also known that there are challenges in producing WSIs of cytological samples; the requirement of Z-stacking to overcome these has been described (Li and Pantanowitz 2022). WSI capture optimisation could be therefore investigated in future studies to improve the analysis of cytology WSIs for our students.

In the current study, the relatively novice PATH2001 students preferred VMI as a study tool over light microscopy, whereas the more experienced MEDI4001 students preferred light microscopy. This is highlighted by feedback received from a MEDI4001 student *"I feel this virtual imager should not replace the on-campus labs as viewing cells under the microscope is a different and vital experience to the virtual imager experience."* A similar disparity between medical students and residents in their preference for light and virtual microscopy has been described (Kuo and Leo 2019), and residents have stated that light microscopy should not be eliminated (Koch *et al* 2009). In line with Kuo and Leo (2019), our study recommends that VM should augment, rather than replace, traditional light microscopy for teaching purposes.

Feedback gained from this study was used to develop version 2.0 of VMI (Figure 2). An information tab has been incorporated to include a brief specimen description. Academics can highlight diagnostic features using an annotation tool, which assists students in visualising normal and abnormal components. This feature is also available to students, allowing them to produce personalised study resources. The inclusions of a scale bar and measurement tool facilitates size comparisons of normal and abnormal cell types. Students can manually mark sections of slides as they view them, so that they have an indication of the areas of the slide analysed. An included differential cell counter allows haematology students to perform white cell differential counts and enables cytology students to experience bronchoalveolar cell counts (BAL). Finally, the issue of not being able to return to the main slide menu was identified by study participants and resolved.

A limitation of our study is that the number of students surveyed is small, particularly those enrolled in MEDI4001. It was not possible to obtain responses from subsequent cohorts due to time constraints during the COVID-19 pandemic and the phasing out of MEDI4001.

In summary, we have developed a robust virtual microscopy platform, VMI, for educational purposes in undergraduate histopathology and cytology. Our WSIs library now consists of over 400 digitised slides, comprising liquid-based cytology samples, FNA smear preparations, histological tissue sections, and a range of blood films for haematology education. VMI is now accessible to students

Figure 2. *Example of VMI, version 2.0. Functionality added in response to student feedback is demonstrated. The hamburger menu in the top left-hand corner (A) allows the user to select specimens and filter them by their unit of study. In the top right-hand corner are buttons that display, from left to right, slide information (B), cell counting functionality (C), a measurement tool (D), annotation functionality (E), and basic tools including full-screen mode (F), image reset (G), settings (H), and help (I). In the bottom left-hand corner is a scale bar in µm (J). The specimen shown is the same field of view as in Figure 1. This image includes an example of a point annotation describing the cells seen (black arrowhead), and an example of the measurement tool (black arrow).*

undertaking the following units delivered in the Curtin Medical School: PATH2001 *(Foundations of Pathology)*, MEDI2002 *(Foundations of Haematology)*, PATH2005 *(Foundations of Anatomical Pathology)*, MEDI3003 *(Principles and Practices of Haematology)*, PATH3004 *(Principles and Practices of Anatomical Pathology)*, and MEDI4010 (*Advanced Anatomical Pathology*), which collectively enrol approximately 300 students per year. In future, we intend to continue to expand our WSIs library in these and other disciplines, such as microbiology, to increase flexibility in learning for future Laboratory Medicine students.

Statements and Declarations

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Appendix 1 – Survey instrument.

Q1 What is your age in years?

- ◯ 18-20 (1)
- ◯ 21-25 (2)
- ◯ 26-30 (3)
- ◯ 31-35 (4)
- ◯ 36-40 (5)
- ◯ 40+ (6)

Q2 What is your gender?

- ◯ Male (1)
- ◯ Female (2)

Q3 Are you a domestic or international student?

- ◯ Domestic (1)
- ◯ International (2)

Q4 I used the imager for study and/or revision purposes in:

- ◯ PATH2001 (1)
- ◯ MEDI4001 (2)

Q5 During the semester, approximately how many digital images did you view using the imager?

- ◯ 1-5 (1)
- ◯ 6-10 (2)
- ◯ 11-20 (3)
- ◯ 21-30 (4)
- ◯ 31-40 (5)
- ◯ 41-50 (6)

Q6 During the semester, approximately how many minutes per week did you use the imager?

- ◯ Less than 10 minutes (1)
- ◯ 10-30 minutes (2)
- ◯ 30-60 minutes (3)
- ◯ 60-120 minutes (1-2 hours) (4)
- ◯ 120-240 minutes (2-4 hours) (5)
- ◯ > 240 minutes (4 hours) (6)

Q7 Use the scale below to indicate your response to the following question; *How useful was the imager for study and/or revision of cytological/histological images? (1)*

- ◯ Extremely useful (1)
- ◯ Very useful (2)
- ◯ Moderately useful (3)
- ◯ Slightly useful (4)
- ◯ Not at all useful (5)

Q8 Did you use the imager on campus?

- ◯ Yes (1)
- ◯ No (2)

Q9 On which of the following devices did you use the imager while on campus? Please select all that apply.

- ◯ Desktop computer in a computer lab (i.e. 308.104) (1)
- ◯ My laptop, connected to the campus Wifi (2)
- ◯ My tablet (i.e. iPad), connected to the campus Wifi (3)
- ◯ My mobile phone, connected to the campus Wifi (4)

Q10 In your opinion, when using the imager on campus, the images loaded:

- ◯ Extremely Slowly (1)
- ◯ Slowly (2)
- ◯ At an acceptable speed (3)
- ◯ Fast (4)
- ◯ Extremely Fast (5)
- ◯ Instantly (6)

Q11 Did you use the imager at home or another off-campus residence?

- ◯ Yes (1)
- ◯ No (2)

Q12 Which type of Internet connection is used at this location?

- ◯ NBN (1)
- ◯ ADSL (2)
- ◯ ADSL2+ (3)
- ◯ Other (4)
- ◯ I don't know (5)

Q13 Do you know the Internet speed at this location?

- ◯ Yes (1)
- ◯ No (2)

Q14 Please type the speed in the box below. (In Mbps; please enter a numerical value only)

____

Q15 On which of the following devices did you use the imager while off campus? Please select all that apply.

- ◯ My desktop computer (1)
- ◯ My laptop, connected to my Wifi (2)
- ◯ My tablet (i.e. iPad), connected to my Wifi (3)
- ◯ My mobile phone, connected to my Wifi (4)
- ◯ My mobile phone, connected to my mobile network (5)

Q16 In your opinion, when using the imager off campus over your home or residential network, the images loaded:

- ◯ Extremely Slowly (1)
- ◯ Slowly (2)
- ◯ At an acceptable speed (3)
- ◯ Fast (4)
- ◯ Extremely Fast (5)
- ◯ Instantly (6)

Q17 In your opinion, when using the imager off campus over your mobile network, the images loaded:

- ◯ Extremely Slowly (1)
- ◯ Slowly (2)
- ◯ At an acceptable speed (3)
- ◯ Fast (4)
- ◯ Extremely Fast (5)
- ◯ Instantly (6)

Q18 Which of the following browsers did you use to access the imager? Please select all that apply.

- ◯ Google Chrome (1)
- ◯ Mozilla Firefox (2)
- ◯ Microsoft Edge (3)
- ◯ Microsoft Internet Explorer (4)
- ◯ Apple Safari (5)
- ◯ Other (6)

Q19 Did you notice any compatibility issues with any of the browsers you used? (i.e. imager didn't open, images didn't load, you couldn't navigate around the image, you couldn't zoom in/out, etc.)

- ◯ Yes (1)
- ◯ No (2)

Q20 Please detail the compatibility issues that you had. Include the browser(s) you were using when you had these issues.

____

Q21 How would you rate the following functionalities of the imager?

Zooming in and out (1)

- ◯ Extremely Poor (1)
- ◯ Poor (2)
- ◯ Neutral (3)
- ◯ Good (4)
- ◯ Extremely Good (5)

Navigating around the image (2)

- ◯ Extremely Poor (1)
- ◯ Poor (2)
- ◯ Neutral (3)
- ◯ Good (4)
- ◯ Extremely Good (5)

Resolution of the image (3)

- ◯ Extremely Poor (1)
- ◯ Poor (2)
- ◯ Neutral (3)
- ◯ Good (4)
- ◯ Extremely Good (5)

Q22 Please rank the following potential improvements to the imager, in the order of importance to you for study and/or revision purposes (Most important feature at the top). Please use the numbered orange box to alter your order.

- ◯ An indication of the slide area that you have viewed already (1)
- ◯ The ability to annotate images yourself (2)
- ◯ The ability to see annotations from your lecturer/tutor/demonstrator (3)
- ◯ The ability to see annotations from your classmates (4)
- ◯ The ability to highlight particular cells or regions of the slide (5)
- ◯ Inclusion of a digital counter for cell counting purposes (6)

Q23 In the space below, please include any additional suggestions for improvements that you think could be made to the imager to improve its use or functionality.

____

Q24 During the semester, which of the following did you use for study and/or revision of cytological/histological images? Please select all that apply.

- ◯ Light microscopy (1)
- ◯ The digital slide imager used in this study (2)
- ◯ Static images, from either lecture notes, a book, or online (3)
- ◯ Another digital slide imager/catalogue available online (4)
- ◯ Video tutorial provided by lecturer/demonstrator/tutor in your unit (5)
- ◯ YouTube/other online video (6)

Q25 Rank the following methods for study and/or revision of cytological/histological images in order of frequency of use (The top method being the one that you use most frequently and the bottom the one used least frequently). Please use the numbered orange box to alter your order.

- ◯ Light microscopy (1)
- ◯ The digital slide imager used in this study (2)
- ◯ Static images, from either lecture notes, a book, or online (3)
- ◯ Another digital slide imager/catalogue available online (4)
- ◯ Tutorial provided by lecturer/demonstrator/tutor in your unit (5)
- ◯ YouTube/other online video (6)

Q26 Rank the following methods for study and/or revision of cytological/histological images in order of benefit (The top method being the one that you found most beneficial for your study and the bottom the one you found the least beneficial). Please use the numbered orange box to alter your order.

- ◯ Light microscopy (1)
- ◯ The digital slide imager used in this study (2)
- ◯ Static images, from either lecture notes, a book, or online (3)
- ◯ Another digital slide imager/catalogue available online (4)
- ◯ Tutorial provided by lecturer/demonstrator/tutor in your unit (5)
- ◯ YouTube/other online video (6)

Q27 Do you have any other comments to make regarding use of the imager? If so, please include them in the space below.

____

____

Appendix 2 – Qualitative survey responses.

In the space below, please include any additional suggestions for improvements that you think could be made to the imager to improve its use or functionality.

PATH2001 Student, Female, 18-20 years of age – "Images labelled with a title rather than just a number to identify them. The magnification factor. Stain used and a key indicating what features they stain (especially for stains we have not encountered before)."

PATH2001 Student, Male, 21-25 years of age – "i do hope that it is possible to name each of the slides rather than number because it makes me confused about what am i seeing for the first time (ex; slide 114 => named as benign prostatic hyperplasia)."

PATH2001 Student, Female, 18-20 years of age – "To label the images with the tissue/organ name and associated disease rather than a number."

PATH2001 Student, Female, 18-20 years of age – "Labelling what tissue is being observed, instead of just a number."

PATH2001 Student, Female, 21-25 years of age – "It would be great to see the slides labelled with the name of the tissue, so that we won't have confusion between similar looking images. Otherwise it's an amazing software, and has helped me out a lot."

PATH2001 Student, Female, 26-30 years of age – "getting some information about the image e.g which tissue, disease .. etc"

PATH2001 Student, Female, 21-25 years of age – "It would be helpful having the name or a descriptor of the tissue for each slide instead of just the number/ code (though the code still works too)"

PATH2001 Student, Female, 36-40 years of age – "only to label slide with tissue and condition/example instead of numbering for easy identification of what was being viewed"

PATH2001 Student, Female, 21-25 years of age – "A discussion board or a note box would be helpful so not only the lecturers could answer questions from students, but students themselves interact and help each other understand images."

PATH2001 Student, Female, 18-20 years of age – "Video summary by lecturer on the same images ie. 'live' (image goes across the window as they describe what is being viewed). Don't know if possible – but video summaries have been very useful for my learning of images."

PATH2001 Student, Female, 40+ years of age – "Maybe throw in some questions for active learning."

PATH2001 Student, Male, 21-25 years of age – "I feel the imager could have a screenshot function or an labelling function. It was mildly annoying having to command-shift-4 and drag the cursor along the field of the image. If a labelling function were to be implemented, it could be good for students to upload labelled images into a folder on blackboard and collaborate with each other as a kind of large study group."

MEDI4001 Student, Female, 26-30 years of age – "I would use the imager more if the images were grouped by headings such as body site (ie. Thyroid cases, Breast cases, Lymph node cases, etc.) instead of grouped by year. I found it impractical to hunt through each module for the relevant slide numbers each time I wanted to review a particular site. It would also be helpful to have each set of slides from a particular case under its own sub-heading as it's a bit confusing when there's slides from other cases right alongside the one you're working on. It would be cool to have the diagnosis/notes/relevant section from the lecture linked on a pane you can click to view as needed. Being able to annotate the image would be a useful feature but there should be an option to view "only lecturer" "only my" "only classmates" or "all" annotations otherwise the slide will very quickly get too cluttered depending on how many people are writing on it. I think this viewer is a huge improvment on Aperio though as not being able to access the old software away from campus stopped me using it as well as the extremely slow loading speed and the frequent tendency for Aperio to crash. This image viewer also provides much higher quality images and is way more user friendly for moving around, zooming in/out and navigating between images. Overall a better program with scope to be a very useful study tool with further funding and development."

MEDI4001 Student, Female, 26-30 years of age – "I found challenges in being able to access cellular details in approximately half of the area for most images due to the image being out focus in those areas. I have no idea how to fix this issue or if there is a program/system which can allow a 3D composite image to be created like is done in a CT and MRI or the interactive anatomy tables (previously used for ISAP). It made it difficult to help assess cells for abnormalities. If annotated images are going to be a feature implemented, then the potential for the original image would be of value to allow students to challenge themselves without the annotation. I feel this virtual imager should not replace the on campus Labs as viewing cells under the microscope is a different and vital experience to the virtual imager experience. Lastly, a name suggestion is InCyte or InCyting view :)"

Do you have any other comments to make regarding use of the imager? If so, please include them in the space below.

PATH2001 Student, Male, 21-25 years of age – "The imager is a good program to use for students to learn cytology or histology at home rather than coming to the lab especially if you don't have the time to do so. I continue to hope the program is used for the next year when students chose cytology or histology as major. I do have some problems regarding waiting time for the images to load so it can have higher resolution."

PATH2001 Student, Female, 21-25 years of age – "With each of the weekly modules I think it would be rather beneficial if the slides had a description of what tissue type we are observing, as well as the number this way it will be certain to know what we will be looking at."

PATH2001 Student, Female, 21-25 years of age – "A great software. Easy to use."

PATH2001 Student, Female, 36-40 years of age – "thank you for all the time taken to compile the images, really helped to consolidate learning, the clarity and zoom in function were excellent and having it available online saved so much time instead of having to visit the lab and view our prepared slides in the microscope. It was an invaluable tool for me this semester."

MEDI4001 Student, Female, 26-30 years of age – "This project should be further funded and developed as it is a very useful tool considering how limited class time is and the reduction of opportunities to review cases one-to-one with lecturers/tutors caused by increased class sizes. Also may be a way for students to ask questions anonymously via student number with the students' names only being viewable to unit coordinator/tutors".

ORIGINAL ARTICLE

Receptor for hyaluronic-acid mediated motility expression as a prognostic/diagnostic indicator in women's cancers

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Abstract

Cancer is known to be the leading cause of death worldwide. Research on cancers that affect women such as breast and ovarian is of high importance due to their sex-specific nature and high mortality rates among women. Early diagnosis of these cancers is a vital component in reducing the rate of mortality. There are many different approaches to diagnosis and biomarkers which can be used to assist in diagnosis. Previous studies have shown a correlation between the increase of RHAMM protein in breast and ovarian cancer. This project aims to examine these studies of RHAMM levels seen in women's cancer tissue and determine if it is a possible biomarker that can be used to diagnose patients. This will be evaluated by performing a systematic review of the current literature.

Keywords: RHAMM, expression, cancer, immunohistochemistry, histology, prognosis

Introduction

Cancer is among the leading causes of death worldwide, contributing to 10 million deaths in 2020 (Romero-Soto *et al* 2021). Due to this alarming statistic, it is of the utmost importance that diagnostic and prognostic techniques undergo continual research to help provide early detection. This systematic review will discuss cancers that affect women such as breast and ovarian due to their sex-specific nature and high mortality rates. It is suggested in a study by Ahn and Jung (2021) that with this result of the significantly high mortality rates, it is pertinent that early detection techniques be developed for these women's cancers.

There are an ever-increasing number of different suggested screening techniques and methods that can be found in the current literature. Due to this enormity, some areas are under-researched begging the question of whether key detection techniques are being missed. Previous studies have shown a correlation between an increase in the protein Receptor for hyaluronic-acid-mediated motility (RHAMM) and the development of several types of cancers such as pancreatic, colon, and multiple myeloma as listed by (Messam *et al* 2021). Therefore, a change in the function of this protein in breast and ovarian tissue could also potentially be indicative of cancer progression suggesting the need for it to be further researched.

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A study conducted by (Messam *et al* 2021, Hinneh *et al* 2022) described RHAMM as a cell surface receptor for hyaluronic acid (HA) which exemplifies a vital role in the cellular stress responses maintaining normal cell growth and survival. HA is a component of the extracellular matrix found in most tissue. As mentioned, it plays a significant role in cell development, wound healing, and regeneration. Due to its role in the cell cycle and function it can promote tumour growth and metastasis when unregulated. HA has cell surface receptors of cluster of differentiation 44 (CD44) and RHAMM, allowing for tumour cells to induce binding to these receptors which can be observed through cellular changes depending on the dose of HA binding. It has been successfully demonstrated by (Lokeshwar *et al* 2014) that these observed cellular changes caused by the binding of RHAMM and CD44 can therefore be used as determinants of tumour growth and metastasis.

RHAMM is particularly vulnerable to changes caused by invasive tumour cells and malignant tissue progression. RHAMM can be expressed both intracellularly and extracellularly with different functions. Intracellular RHAMM is responsible for the regulation of mitotic spindle and microtubule formation, whereas extracellular RHAMM observes a HA receptor role, inducing cell migration in response to inflammation, and inducing wound healing (Messam *et al* 2021; Hinneh *et al* 2022).

The RHAMM gene consists of 18 exons that express various isoforms, produced through the splicing of mRNA. RHAMM expression is low in normal tissue, however, in the presence of tissue injury, it is subsequently increased. These spliced isoforms of RHAMM are expressed in cancers such as multiple myeloma, breast, pancreatic, and colon cancers (Messam *et al* 2021). RHAMM has an oncogenic potential in its intracellular function due to its ability to form mitotic spindles and its extracellular role in cell migration. Specifically in breast cancer, RHAMM overexpressed, possessing key roles in several molecular signalling pathways such as the MAPK (ERK1/2) pathway which allows for protein communication with DNA in the nucleus of a cell, increasing cell cycle progression (Hinneh *et al* 2022).

Currently the literature suggests that genetic or pharmacological manipulations of proteins such as RHAMM in tumour tissue are possible therapeutic targets. In studies over the last 10 years, it has been observed that HA fragments of RHAMM and CD44 are identified in the urine of patients with bladder cancer and the saliva of patients with head and neck cancer, further exemplifying its possibility to be used as a diagnostic target. It can furthermore be proposed that the silencing of RHAMM and CD44 receptors could be a potential cancer therapy target (Lokeshwar *et al* 2014). RHAMM is also over-expressed in tumour cells and its pro-tumour activity is dependent on its subcellular distribution, further increased upon inflammatory stimuli making it a difficult target for cancer therapies but one that should be considered when it comes to diagnosis and prognosis. RHAMM overexpression is generally associated with metastatic and aggressive phenotypes of cancer epitomising its potential use as a biomarker for diagnosis and prognosis (Messam *et al* 2021; Hinneh *et al* 2022).

This project aims to systematically review the use of RHAMM in the diagnosis and prognosis of women's cancers specifically ovarian and breast. This will be achieved by reviewing literature from the last 10 years which discuss the role of RHAMM in cancer development and how it can be screened via the use of immunohistochemistry staining to identify levels that may provide diagnostic or prognostic indicators for patients. Comparisons will be made between the articles to determine if RHAMM is a possible diagnostic and prognostic indicator for early diagnosis of women's cancers.

Materials & methods

Due to the large volume of cancer articles, a systematic review and critical appraisal of the current literature was conducted to determine which articles were of value to the aim of this report. This systematic review used statistical methods of meta-analysis using Covidence a systematic review tool following the PRISMA guidelines as stated by (Moher *et al* 2009). The eligibility of the articles used was formulated using the PICOS search strategy.

Eligibility criteria

Through the collection of articles into EndNote, these articles were imported into Covidence, an online systematic review screening tool. The screening process followed the below PICOS framework for systematic reviews.

(P) Population: Studies outlining RHAMM expression in ovarian and breast cancer are to be included. All other types of cancer are to be excluded. All ages of patients and locations of research are to be included.

(I) Intervention: Studies assessing the molecular mechanisms of RHAMM expression in breast and ovarian cancers along with their diagnostic and prognostic factors to be included. Studies looking specifically at the post-chemotherapy/ drug interaction properties of these biomarkers are to not be included.

(C) Comparison: RHAMM identification techniques and suggested levels/ quantity relevant for diagnosis or prognosis.

(O) Outcome: The studies to be included should discuss the potential for the use of RHAMM as a biomarker in the diagnosis and prognosis of ovarian and breast cancers and their relation to possible therapeutic or diagnostic targets.

(S) Study design: All types of studies to be included e.g. qualitative, quantitative, or mixed mode.

Other: Studies published in English only to be included. Studies relevant to humans are only included. Studies from the last 10 years are to be included (2013-2023) to include the most up-to-date statistics and quantitative parameters. All duplicate studies or without full text/ DOI reference numbers are to be excluded.

Information sources

This systematic review comprises articles searched from 3 specific databases selected based on their accessibility and productibility of articles related to medicine, health, and science:

- PubMed
- Scopus
- Embase

No search filters or limitations were applied during initial research on these databases.

Search strategy

The search strategy for this systematic review was based on key terms which are relative to the topic of interest. These key terms were filtered through the preliminary searches to allow for a collection of relevant articles. Key terms included RHAMM, expression, cancer, immunohistochemistry, diagnosis, and their relevant synonyms as seen in Table 1. The internal EndNote PubMed database was used in selecting criteria of 'all fields' and the 'and' 'or' selection when formulating the search. When using Scopus and Embase their direct websites were used using a phrase search technique to produce the most accurate results. "(RHAMM expression in cancer) OR (Receptor for hyaluronic acid-mediated motility) OR (CD44) AND (progression) AND (immunohistochemistry) AND (histology) AND (prognosis)".

All articles were compiled in EndNote and then exported into Covidence to allow for systematic reviewing to be completed.

Data collection

Data was managed using EndNote software. The internal web base was used in conjunction with electronic internet databases to extract and import data to EndNote. The final selected articles were then extracted from Covidence which is the preferred systematic review tool.

Risk of bias and quality of studies

The JBI Critical Appraisal Checklist tool produced by (Aromataris, 2023) was used to assess the quality of the studies chosen. This tool uses ten specific questions to determine if the articles which are chosen to be included in this systematic review provide methodological quality and if the study has accurately assessed the possibility of bias in the way the report has been designed, conducted, and analysed. Through answering 'Yes', 'No' or 'Unclear' studies are then deemed eligible if they provide a 'Yes' answer for 70% or more of the questions. Those that do not meet this criterion were still included to make comparisons as seen in Table 2.

Study selection

- Stage 1: Studies found through the search strategy underwent title and abstract screening as a part of the Covidence systematic review tool program. Studies that were not relevant to the inclusion criteria were removed if they refer to animal research. Additionally, duplicates were removed by the Covidence internal system before entering the Title and Abstract screening.
- Stage 2: The articles left after Stage 1 were entered into the full-text review. Where they were removed if they were not within the last 10 years. Articles not available in full text or with a DOI were also removed. Studies not targeted to specifically RHAMM were also removed. Additionally, studies not specific to breast or ovarian cancer were excluded.
- Stage 3: Studies that were passed were included in the systematic review following a quality and BIAS examination through JBI Critical Appraisal Tool.

Search results

The search strategy identified 2127 articles. Once screening the titles of these articles 1723 were removed as they were irrelevant to the research aim. 308 articles then underwent full-text studies to determine their relevance. 304 of these articles were excluded from the systematic review. 131 articles were not in the last 10 years. 128 were the wrong patient population e.g. referring to animal studies and or other types of cancers not specific to ovarian or breast. 22 studies were not discussing the use of RHAMM specifically as a biomarker. 20 studies were of the wrong design not providing immunohistochemistry or flow cytometry results. 3 articles did not provide a full text which could be accessed. A PRISMA diagram outlining the study selection mentioned was produced using the systematic tool program 'Covidence' as seen in Figure 1 (Moher *et al* 2009). The remaining 4 articles underwent bias appraisal using the JBI Critical Appraisal checklist as seen in Table 2.

Selected studies

- Buttermore *et al* 2017 - Increased RHAMM expression relates to ovarian cancer progression
- Carvalho *et al* 2022 - RHAMM expression tunes the response of breast cancer cell lines to hyaluronan
- Veiseh *et al* 2014 - Cellular heterogeneity profiling by hyaluronan probes reveals an invasive but slow-growing breast tumour subset
- Juan Wang *et al* 2020- RHAMM inhibits cell migration via the AKT/GSK3β Snail axis in Luminal A subtype breast cancer

Critical appraisal of study bias and quality

Four articles were examined using the JBI critical appraisal checklist as seen in Table 2. If a result of <70% is obtained using the JBI critical appraisal tool it should be excluded from the systematic review, however in this case these articles will still be used based of their relevance. Two articles utilised breast cancer cell lines to be analysed using flow cytometry, therefore were no direct patient results that could be compared. These studies used the standardised cell lines which are often used in breast cancer research therefore the quality of these results can be assumed to be accurate and were used considering possible limitations (Veiseh *et al* 2014; Carvalho *et al* 2022).

Study Design

The key information collected from the four chosen articles were compared and this is outlined in Table 3. All four articles investigated the increase in RHAMM levels in association with different types of women's cancers. Two of these articles did so by immunohistochemistry of patient cancer surgical tissue specimens. The other two articles utilised known aggressive breast cancer cell lines via flow cytometry as seen in Figures 2 and 3. These breast cancer cell lines included MDA-MB-231 which is basal mesenchymal, triple-negative breast cancer and Sk-Br-3 which is HER2-positive breast cancer. MDA-MB-231 in particular is a highly aggressive type of breast cancer which is why it was selected for this particular study (Carvalho *et al* 2022; Veiseh *et al* 2014).

Table 4 compares the histological techniques used in two of the articles. One article uses breast cancer tissue biopsies, the other uses surgically removed epithelial ovarian cancer and low malignant potential tumours. Both articles used a similar type of anti-RHAMM antibody. Both articles used the same tissue counterstain methods and similar fixation techniques (Buttermore *et al* 2017; Wang *et al* 2020).

Results

Table 1. *Example search terms and synonyms used for search strategy.*

Search Term	Synonyms
RHAMM	Receptor for hyaluronic acid-mediated motility, CD44
Expression	progression
Cancer	tumour
immunohistochemistry	histology
Diagnosis	prognosis

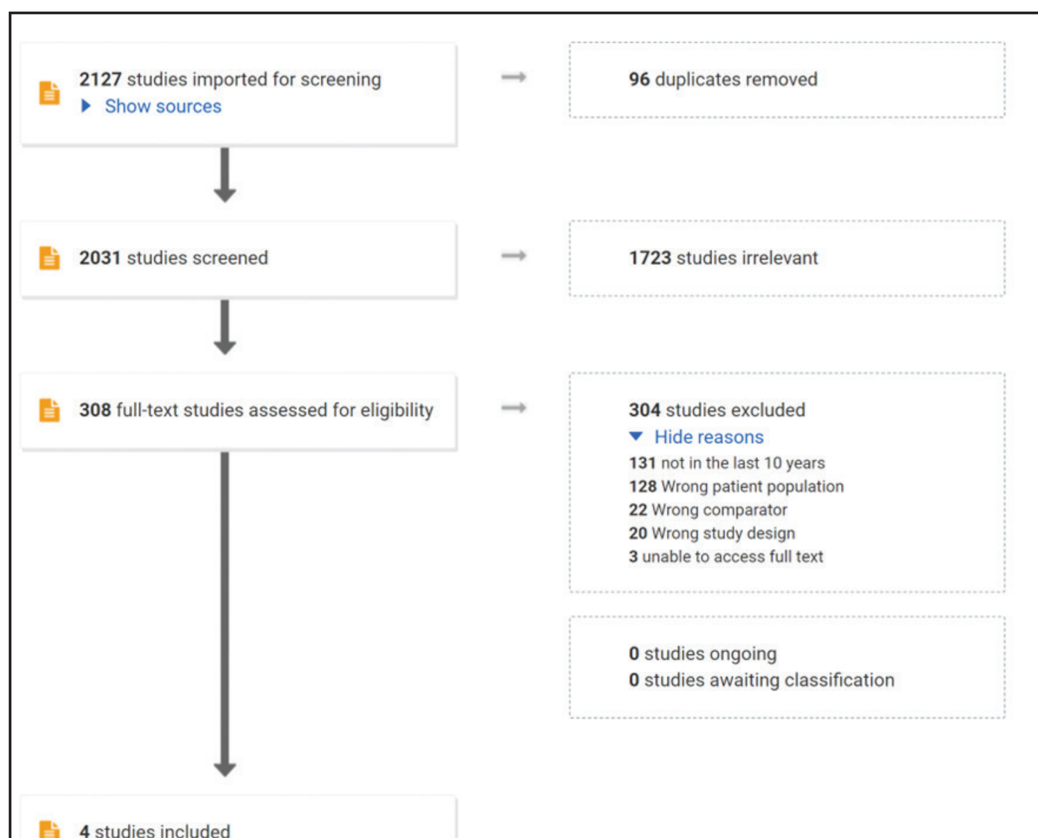

Figure 1. *Prisma diagram from Covidence Systematic Review Tool*

Table 2. *JBI Critical Appraisal Checklist*

	(Veiseh *et al* 2014)	(Buttermore *et al* 2017)	(Carvalho *et al* 2022)	(Wang *et al* 2020)
1. Was a consecutive or random sample of patients enrolled?	Yes	Yes	Yes	Yes
2. Was a case-control design avoided?	No	No	No	No
3. Did the study avoid inappropriate exclusions?	n/a	Yes	n/a	Yes
4. Were the index test results interpreted without knowledge of the results of the reference standard?	Yesv	Yes	Yes	Yes
5. If a threshold was used, was it pre-specific?	n/a	n/a	n/a	n/a
6. Is the reference standard likely to correctly classify the target condition?	Yes	Yes	Yes	Yes
7. Were the reference standard results interpreted without knowledge of the results of the index test?	Yes	Yes	Yes	Yes
8. Was there an appropriate interval between the index test the and reference standard?	n/a	Yes	n/a	Yes
9. Did all patients receive the same reference standard?	n/a	Yes	n/a	Yes
10. Were all patients included in the analysis?	n/a	Yes	n/a	Yes
Percentage of 'Yes' answers (included or excluded)	40% included	80% included	40% included	80% included

**4 articles were examined using the JBI critical appraisal checklist, typically if a result of <70% is obtained using the JBI critical appraisal tool it should be excluded from the systematic review, however in this case these articles will still be used based on their relevance.*

Table 3. *Data extraction table comparing articles*

Author and publication year	Cancer	Biomarker assessed	Location of study	Type of testing methods used	Number of participants	Results and conclusion
(Buttermore *et al* 2017)	Ovarian cancer	RHAMM	Florida, US	Immunohistolo-gy-chemistry	36	RHAMM is overexpressed in 91% of clinical specimens of OC. RHAMM staining was most intense among clinically aggressive OC histologic subtypes.
(Carvalho *et al* 2022)	Breast cancer	RHAMM, CD44	Minho, Portugal	Immunofluores-cence and cyto-chemistry	n/a breast cancer cell lines used	40% Sk-Br-3 cells positive for cell surface RHAMM. 5% MDA-MB-231 cells RHAMM positive. Higher average expression of RHAMM was observed for MDA-MB-231 cells than Sk-Br-3.
(Wang *et al* 2020)	Breast cancer	RHAMM	China	Immunocytochem-istry/ Immunohis-tochemistry	155 (124 malignant breast cancer tissues, 31 normal/ cancer adjacent tissues)	RHAMM expression is increased in malignant breast cancer versus normal/ cancer adjacent tissue. RHAMM intensity Grade 0- 0-25%, Grade 1- 26—50% (light brown), Grade 2- 51-75% (intermediate brown) and Grade 3- 76-100% (dark brown)
(Veiseh *et al* 2014)	Breast cancer	RHAMM, CD44	California, US	flow cytometry	n/a breast cancer cell lines used	Correlation between high levels of RHAMM and aggressive MDA-MB-231 cell line

**N/a for cancer cell lines used instead of patient tissue results in reports that utilized flow cytometry to analyse RHAMM expression in comparison to immunohistochemistry.*

Table 4. Comparison of histological techniques and IHC staining

Author and publication year	Method of immunobiological analysis	Counterstain used	Tissue collection method	Tissue fixative method
(Wang *et al* 2020)	Anti-RHAMM antibody (Sigma, Aldrich St. Louis, MO) (Anti-CD168/ RHAMM antibody produced in rabbit) at 4 degrees Celsius overnight.	Haematoxylin.	Human breast tissue microarray. 124 malignant breast cancer tissue and 31 normal/cancer adjacent tissue from breast cancer patients.	4% Paraformaldehyde. Paraffin embedded.
(Buttermore *et al* 2017).	Rabbit anti-human CD168 primary antibody (1:100) (CA#:PA5-32309 ThermoFisher Scientific, Waltham, MA)	Haematoxylin and mounted with Lecia Micro mount	Primary surgery with complete surgical staging or epithelial ovarian cancer (EOC) and low malignant potential tumours (LMP) is defined as benign with abnormal cells.	10% formalin-fixed. Paraffin-embedded.

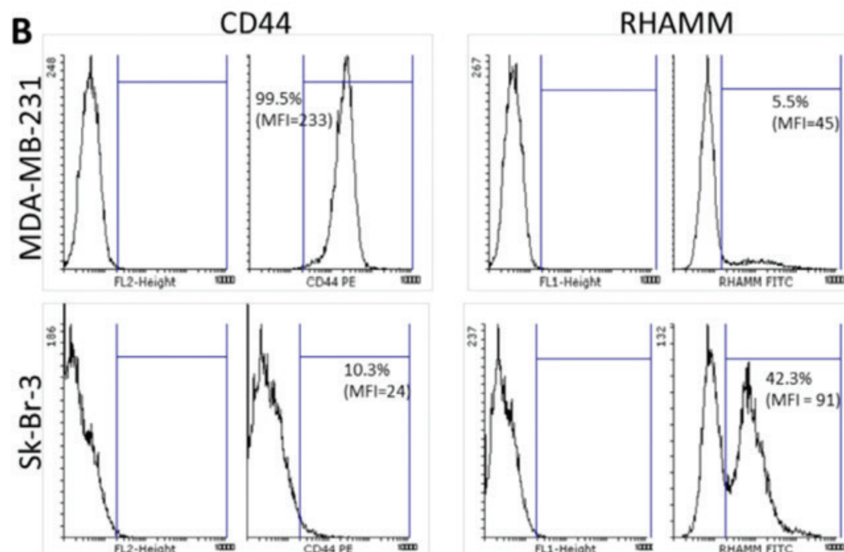

Figure 2. *Flow cytometry of RHAMM and CD44 expression on the surface of breast cancer cell lines MDA-MB-231 and Sk-Br-3. 42.3% Sk-Br-3 cells positive for cell surface RHAMM. 5.5% MDA-MB-231 cells RHAMM positive (Carvalho* et al *2022).*

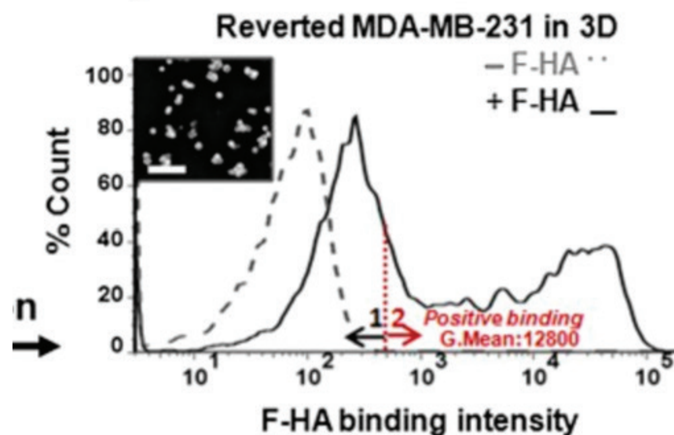

Figure 3. *Cell surface RHAMM binding to Fluorescent Hyaluronan (F-HA) in MDA-MB-231 breast cancer cell line (Veiseh* et al *2014)*

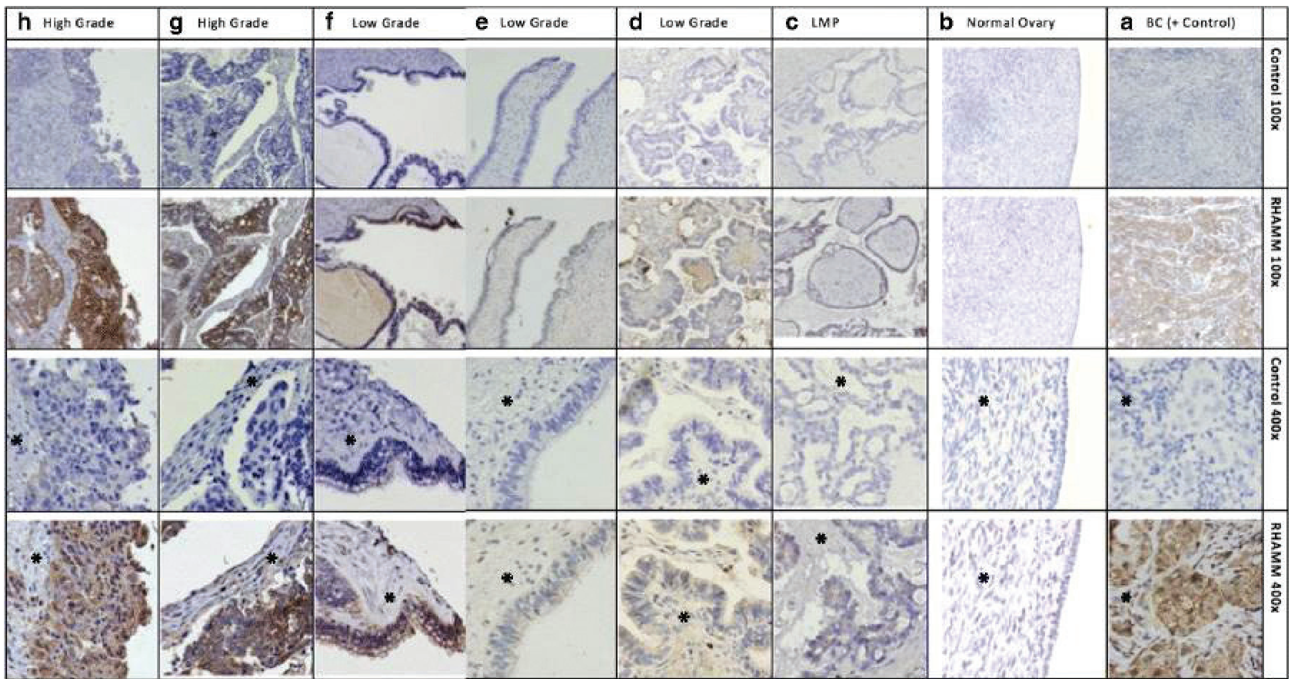

Figure 4. *Immunohistochemistry staining of ovarian cancer tissue specimens at 100x and 400x magnification. Immunostain rabbit anti-human CD168, primary antibody (1:100) used. (A) Control breast cancer tissue was seen with normal H&E staining and tissue was treated with antibodies to view the presence of RHAMM. (B) Normal ovary tissue with H&E staining and tissue treated with antibodies. (C) Low malignant potential tumour ovarian cancer tissue with H&E staining and with antibody treatment to observe RHAMM. (D, E, F) Low-grade ovarian cancer tissue with H&E staining and antibody treatment. (G) High-grade breast cancer was used as a control with H&E and antibody staining. (H) High-grade ovarian cancer tissue with H&E staining and treated with antibodies to identify RHAMM. Staining is described as Weak <30%, moderate 30-50%, and strong >50% (Buttermore* et al *2017).*

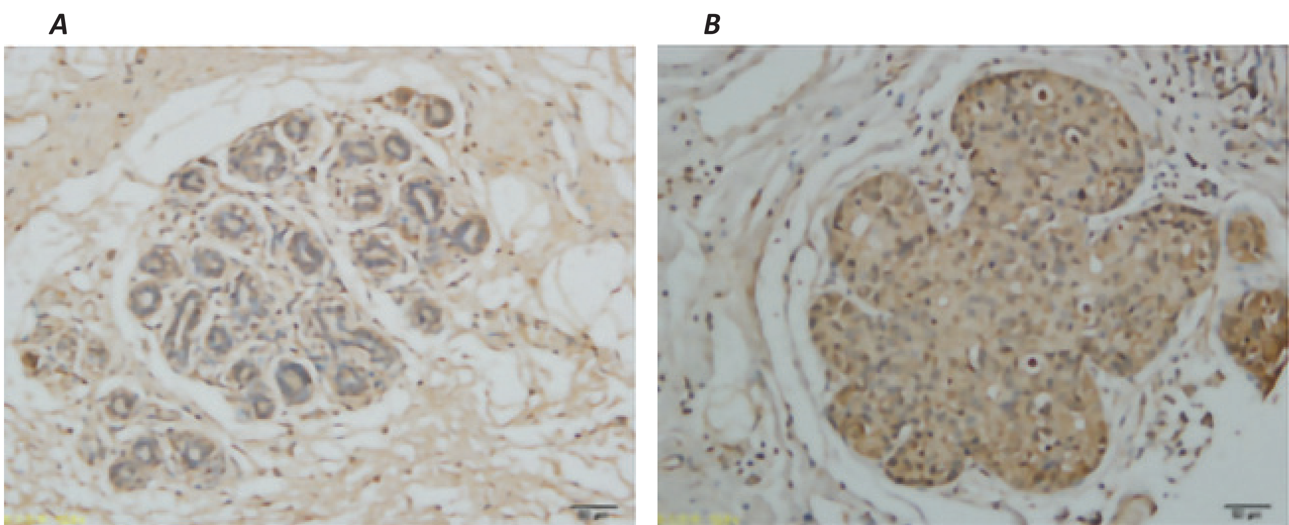

Figure 5. *Immunocytochemistry breast cancer tissue at 200x magnification. Immunocytochemical staining of normal/cancer adjacent breast tissue (A) vs malignant breast tissue displaying increase in RHAMM expression (B). RHAMM intensity Grade 0- 0-25%, Grade 1- 26—50% (light brown), Grade 2- 51-75% (intermediate brown) and Grade 3- 76-100% (dark brown) (Wang* et al *2020).*

Discussion

The current study systematically reviewed published articles discussing RHAMM expression in women's cancers specific to breast and ovarian cancer. The current literature was searched following the strict eligibility criteria and four articles were selected to be analysed in this systematic review (Figure 1). The four articles all report an increase in the level of RHAMM across breast and ovarian cancers as seen in Table 3 (Veiseh *et al* 2014; Buttermore *et al* 2017; Wang *et al* 2020; Carvalho *et al* 2022). These results support the aim and suggest that RHAMM could be used as a prognostic or diagnostic factor in women's cancers, duplicating previous studies where it is used to diagnose other types of cancers. The findings of this study display that increased RHAMM levels in patient cancer tissue and cells can be a possible diagnostic biomarker, allowing for early diagnosis and prognostic determination.

RHAMM was researched in breast cancer cases with flow cytometry used in two breast cancer studies to determine the presence and significance of RHAMM in breast cancer cell lines. Both articles outlined the breast cancer cell line MDA-MB-231. One article also discussed another breast cancer cell line Sk-Br-3. As seen in the flow cytometry image Figure 2 RHAMM is expressed on 42.3% of Sk-Br-3 cells and 5.5% cell surface expression on MDA-MB-231 cells (Carvalho *et al* 2022). This relation to increased RHAMM levels is also seen in one other article which discusses the use of MDA-MB-231 the most malignant breast cancer cell line. As seen in Figure 3 using flow cytometry, a high presence of RHAMM levels binding to F-HA on the cell surface was observed (Veiseh *et al* 2014). This presence of RHAMM concerning aggressive breast cancer cell lines again suggests the importance of using RHAMM as a prognostic indicator of cancer and also a possibility for diagnostic use.

Two studies displayed an increase in RHAMM levels in cancer tissue specimens using immunohistochemistry techniques with antibodies saining the malignant cells. As seen in Table 4, both studies utilised a group of patient participants who had undergone surgical removal of cancerous breast tissue and ovarian tissue. This tissue in both studies was fixed with similar formaldehyde solutions, paraffin-embedded, sectioned and stained using haematoxylin and eosin (H&E) staining. They both used a similar type of rabbit antibody when performing immunohistochemistry/immunocytochemistry staining, which suggests that these particular rabbit antibodies are effective at staining RHAMM in tissue (Buttermore *et al* 2017; Wang *et al* 2020).

RHAMM levels in the histological tissue were seen in Figures 4 and 5. A positive control breast cancer (BC) tissue was used in one article to make comparisons to the ovarian cancer tissue as seen in Figure (A). The BC control was described as 'intense, punctate/diffuse cytoplasmic staining' (Buttermore *et al* 2017) which was consistent amongst the cytoplasmic staining seen in the ovarian tissue in Figure 2 (C, D, E, F, H). In this study 22/36 patients presented serous ovarian cancer (OC) (epithelial ovarian cancer). Serous OC displayed 91% RHAMM staining in the cytoplasm ranging from weak <30%, moderate 30-50%, and strong >50% (Buttermore *et al* 2017). As seen in Figure 4 (D, E, F) low-grade serous cancer (LGSC) had a calculated 14% (weak) RHAMM staining intensity. Comparatively, as seen in Figure 4 (G, H) high-grade serous cancer (HGSC) had a calculated RHAMM staining intensity of 53% (strong). These results suggest that RHAMM staining intensity is correlated with the stage of cancer indicating a potential prognostic indicator (Buttermore *et al* 2017). Another study also displayed similar results of correlation between RHAMM expression and more aggressive serous carcinomas.

This can be seen in Figure 5 in the immunocytochemistry microscopy displaying the presence of RHAMM in varying scores in malignant breast tissue and normal/adjacent breast tissue. RHAMM intensity is determined as Grade 0- 0-25%, Grade 1- 26—50% (light brown), Grade 2- 51-75% (intermediate brown) and Grade 3- 76-100% (dark brown). As displayed in Figure 5 A normal/ adjacent breast tissue samples displayed low grade 0-1 RHAMM staining whereas in Figure 5 B malignant breast tissue RHAMM staining is increased and graded as 2-3 (51-100%). These findings further support the suggestion that RHAMM is more prevalent in aggressive cancer types and could be used as a prognostic indicator (Wang *et al* 2020).

With the use of different diagnostic techniques and different biological samples of patient surgical cancer tissue samples and established cancer cell lines, all four studies were able to display a correlation between increased RHAMM levels in women's cancers. All articles not only display an increase in RHAMM but also a relationship between the cancer stage and aggressiveness.

Reliability of results

As discussed previously, two of the articles were included even though they used breast cancer cell lines rather than actual patient samples.

Whilst the other two studies received 80% on the JBI critical appraisal these studies did not provide patient details such as age, ethnicity, or prior medical history in relation to the specimens analysed, which may be a potential factor that limits the accuracy of the results recorded (refer to Table 3) (Wang *et al* 2020; Buttermore *et al* 2017). They did, however, utilise similar tissue fixative and counter-staining methods along with the use of similar antibodies

to achieve RHAMM staining (Table 4), which is standard practice in immunohistochemistry/immunocytochemistry suggesting that due to standardised testing, the results can be assumed to be accurate when adhering to the same testing procedures (Wang *et al* 2020; Buttermore *et al* 2017).

Limitations and future directions

There are limitations in this systematic review. The most prevalent is that there was only one article discussing RHAMM expression in breast cancer using immunohistochemistry. Following the search criteria there were no other relevant articles in the literature which discuss the use of RHAMM in immunohistochemistry and produced histological microscopy images to make a comparison. This may be attributed to the fact that there are already many diagnostic techniques and biomarkers which have been developed and proven to be successful indicators of breast cancer, however, that does not signify RHAMM could provide further diagnostic or prognostic assistance.

Secondly as previously mentioned, RHAMM is researched in other types of aggressive and metastatic cancers such as pancreatic, colon and multiple myeloma and only these few articles delve specifically into RHAMM expression in women's cancers such as ovarian and breast (Hinneh *et al* 2022; Messam *et al* 2021). This lack of general research makes the connection between the increase of RHAMM and its correlation to aggressive women's cancers difficult. This systematic review cannot confirm that increased RHAMM expression can provide a diagnosis or a prognostic indication for cancer patients, but this is an indicator that future research should be invested into this correlation. Further studies utilising surgically removed tissue from diagnosed cancer patients should be examined using immunohistochemistry to further determine the relationship between RHAMM and cancer stage.

As mentioned, two studies did not provide patient details such as age, ethnicity, or prior medical history in relation to the specimens analysed which could be a potential factor that limits the accuracy of the results. In future studies utilising patient tissue these patient cohort characteristics should be evaluated to provide more thorough reliable data. Furthermore, these studies that evaluated RHAMM levels in the tissue did not give specific percentages for RHAMM levels in each tissue type, only providing a generalised Grade 0- 0-25%, Grade 1- 26—50% (light brown), Grade 2- 51-75% (intermediate brown) and Grade 3- 76-100% (dark brown) grading of RHAMM staining, suggesting that further studies conducted need to accurately determine quantitative levels to allow for better scientific comparisons to be made.

Finally, as these tissue specimens and cell lines used were from patients who have already been diagnosed with cancer, it is difficult to determine if RHAMM levels could be observed in histological specimens from patients prior to cancer diagnosis. That is why other diagnostic tests such as flow cytometry were discussed to allow for other methods in conjunction with immunohistochemistry to provide more thorough research.

Overall the findings of this systematic review do suggest that there is a significant correlation between increased RHAMM expression and women's cancers. Extensive further research is therefore required to establish whether RHAMM could be a key biomarker in the prognosis and potential diagnosis of women's cancers. Future studies should aim to evaluate RHAMM levels in breast cancer tissue through further immunohistochemistry and determination of quantitative significant levels of RHAMM in patient populations.

Conclusion

All four articles examined in this systematic review found a correlation between increased RHAMM levels and women's cancers, particularly those which are highly aggressive and metastatic. This finding is consistent with the findings of other reported studies of increased RHAMM levels in other types of aggressive cancers in the literature. This indicates a substantial need for further research into the use of RHAMM as a biomarker of immunohistochemistry tissue staining and/ or in conjunction with other diagnostic tests such as flow cytometry to assist in the prognosis evaluation and diagnosis of patients. Limitations of this study and previous studies should be taken into account when further researching the prevalence of RHAMM to collect accurate data which can be used to further define significant quantitative levels of RHAMM to assist in providing diagnoses and prognostic indicators to reduce the burden of women's cancers.

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ORIGINAL ARTICLE

Insights into continuing professional development within higher education

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Abstract

Clinical placement supervisors and medical laboratory science (MLS) academics play a key role in nurturing the 'work-ready' skills of MLS students. The currency of learning content has a significant impact on professional competency and graduate learning attributes. This study aimed to (a) understand the perceptions of clinical placement supervisors regarding the clinical and professional competency of current students and graduates of the Australian Institute of Medical and Clinical Scientists (AIMS) accredited MLS programs; and (b) to explore its potential link with the continuing professional development (CPD) and currency of academics teaching into accredited programs. The study was conducted in two phases. Two anonymous online surveys designed using SurveyMonkey were distributed to clinical placement supervisors (Phase 1) and teaching academics of AIMS-accredited MLS programs (Phase 2). Likert scale type, demographic, open and closed-ended questions were employed. The study highlighted the importance of currency and CPD of academics in delivering high-quality, professionally relevant learning activities.

Keywords: ***Continuing professional development, medical laboratory science, Australian Institute of Medical and Clinical Scientists clinical placement, academic development, APACE***

Introduction

Medical laboratory science (MLS) programs in Australia and overseas that are accredited by the Australian Institute of Medical and Clinical Scientists (AIMS) adhere to standards and requirements set out by AIMS to maintain professional accreditation. The requirements represent current learning and professional practice standards for entry-level (Bachelor programs), graduate and advanced-level (Master programs) medical scientists. While accreditation standards embody thorough and extensive requirements, the dynamic nature of the profession necessitates accredited university programs to actively engage with evolving updates and developments. Regular updates to learning content, assessment framework and teaching styles are essential to ensure currency with professional demands of graduate medical scientists (Graffam 2007).

Continuing professional development (CPD) plays a key role in improving the overall quality of patient care and is increasingly considered essential in the medical laboratory workforce (Wilson and Badrick 2016). It is interesting to note that employment as a tertiary educator in Australian and international universities does not require a formal education qualification or a minimum number of CPD hours to ensure maintenance of high-quality contemporary pedagogical practice (Weuffen *et al* 2020). The competency-based standards of AIMS clearly outline in Unit 6 the expectations of professional accountability and participation in CPD for Australian medical scientists. It is essential for academics actively teaching in an AIMS-accredited program to maintain currency within their profession to meet strict educational, medical, and clinical standards. Knowledge and skills acquired by academics during CPD are ideally passed down to students they teach so that graduate attributes and graduate learning outcomes, which include communication, professional practice, ethics, lifelong learning, and global citizenship are met. Issues can however arise relating to the content and focus of CPD, differences pertaining to what constitutes a CPD activity, and the relationship between institutional and individual interests, policy, and implementation (Crawford 2008).

In addition to MLS academics, clinical placement supervisors play an important role in contributing towards graduate learning outcomes. Work-integrated learning (WIL) provides hands-on experience and

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prepares students for ‘real-world’ experiences. Such experiences also provide students the opportunity to reflect on and apply skills acquired during their program learning to ‘on-the-job’ tasks. Placement supervisors and WIL academics work collaboratively in administering, monitoring progress, and supporting students through their placement. The currency of learning content has a significant impact on student learning experience, performance, and graduate opportunities (Closs *et al* 2022). This is considered advantageous to professional stakeholders who benefit from being involved directly in building skills of graduates relevant to the workplace and to the university in promoting graduate employability (Lowden *et al* 2011).

The research presented herein investigated: 1) the perceptions of clinical placement supervisors regarding clinical/professional competency of current students/graduates of AIMS-accredited MLS programs; and 2) explored a potential link with CPD and currency of academics teaching into AIMS accredited programs.

Methods

This study was approved by the Charles Sturt University Human Research Ethics Committee (Protocol number – H22259). All participants provided informed consent before participating in the study.

The study was conducted in two phases. Phase 1: the primary objective was to understand the current situation regarding currency and competency of graduates and/or current students at AIMS-accredited MLS programs. This information was used to postulate specific criteria and standards for phase 2 of the project. An anonymous online survey (Supplementary 1) designed through Survey Monkey was advertised to clinical placement providers. The questionnaire intended to draw key information that would help assess the current standards of program delivery and identify areas of improvement (particularly in core clinical departments) that might potentially be linked to the currency of academic staff.

Phase 2: to evaluate the current CPD activities undertaken by academics teaching in AIMS-accredited programs, a detailed questionnaire (Supplementary 2) listing all possible types of CPD activities was advertised. The questionnaire consisted of CPD activities that are aligned with the Pathology Associations Council’s Medical Scientist competency standards and the AIMS Australasian Professional Acknowledgement of Continuing Education (APACE) program. Academics were requested to select all possible CPD activities listed in the questionnaire. Demographic data such as teaching experience, qualifications, academic level, and subject area were also collected.

Results

Phase 1: Twelve responses were received from placement providers who play a key role in supervising students on clinical placements. Their roles ranged from laboratory/operations manager, scientists, and laboratory supervisor with 66% from public laboratories and 33% from private laboratories. The majority of the supervisors belonged to a multidisciplinary laboratory (9 responders) with two from single discipline laboratories and one from a short turnaround time (STAT) laboratory. All providers indicated that they had hosted a student less than a year ago. Eighty-three percent% of the responses highlighted that a key factor for a laboratory’s willingness to host students for placement would depend on capacity to manage extra work demands on supervising staff and 92% indicated the availability of staff to supervise the students as another key factor. Important to note is that 42% of the supervisors highlighted student competency as a key factor for a laboratory’s willingness to accommodate a student for clinical placement.

Table 1. *Teaching experience of academic staff surveyed*

Answer choices	Responses	Count
1 year or less	16%	4
2 - 5 years	19%	5
6 - 10 years	23%	6
11 - 20 years	27%	7
More than 20 years	15%	4
Total		26

Table 2. *AIMS membership levels of academics surveyed*

Answer choices	Responses	Count
Fellow	12%	2
Full - Professional	23%	4
Graduate	12%	2
Member - Non-specific discipline	23%	4
Member - Discipline-specific	18%	3
Research	0%	0
Retained	0%	0
Non-voting - Affiliate	12%	2
Non-voting - Student	0%	0
Total		17

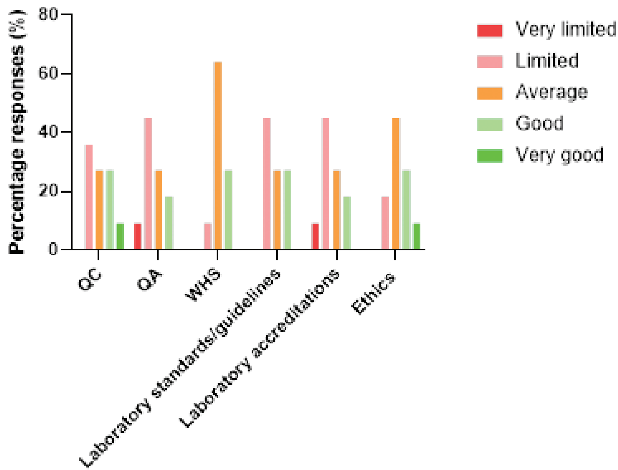

Figure 1. *Clinical supervisors' perception of student's understanding of key concepts in the workplace. QC, quality control; QA, quality assurance; WHS, workplace health and safety.*

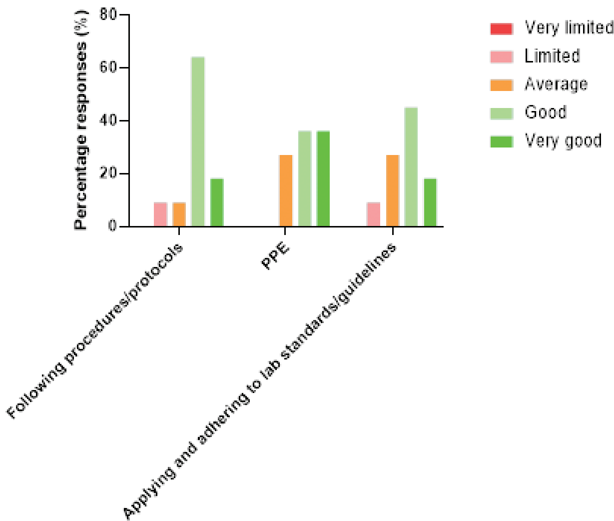

Figure 2. *Clinical supervisors' perception of student's knowledge of laboratory practices. PPE, personal protective equipment.*

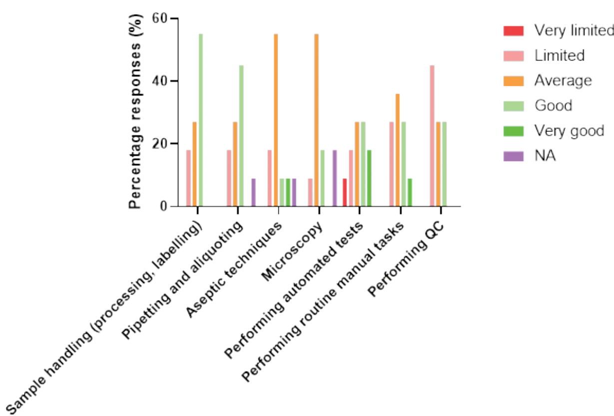

Figure 3. *Clinical supervisors' perception of student's hands-on skills in the workplace*

Figure 1 illustrates students' understanding of key concepts in the profession according to the placement supervisors. Figure 2 represents student knowledge of laboratory practices. The survey highlighted that overall students had a 'good' knowledge of sample handling including pipetting and aliquoting but emphasised a deficiency in adhering to aseptic techniques and microscopy. One of the key skills that was noted that students lacked was performing quality control testing (Figure 3).

The survey results indicated that students on average integrated well in areas of the workplace such as teamwork, punctuality, ability to follow directions, communication and interpersonal skills, willingness to comply with directions and responsiveness to feedback. Written responses in the survey indicated the need for currency in instrumentation and an understanding of common laboratory errors (clerical and pre-analytical). It was also noted by the placement supervisors that quite often at the end of the student's clinical placement, there is a potential offer of employment (45% response).

Phase 2: Twenty-six survey responses were received from academics teaching in AIMS-accredited programs. Sixty-five percent of the academics had a PhD qualification, 27% had a Master degree and the rest of the responders completed a Bachelor degree. The teaching experience of all academic staff is presented in Table 1. Sixty-five percent (17 out of 26) of the academics were members of AIMS with levels of membership presented in Table 2.

Most of the academics (80%) indicated their membership with other professional bodies such as AACB, RCPA, ASI, IBMS among others, while 88% of the academics did not hold a current APACE certificate offered by AIMS and 91% indicated they have not previously held this certification. Seventy-three percent of the academics indicated that they actively engage in peer reviewing journal articles with 32% indicating 1-2 reviews per year, 26% indicating 3-5 reviews per year and 42% reviewing more than 5 articles per year. Figure 4a indicates the CPD activities the academics engaged in over the last three years and 4b highlights the usefulness of the CPD activities that have contributed towards their teaching activities.

It was also highlighted that the knowledge or skills gained from the CPD activities were utilised and incorporated into lectures, course materials, tutorials, or practicals. Forty-seven percent of the responses noted that the CPD activities and associated knowledge added value to teaching. Academics noted that student academic performance reviews, quizzes, case studies and group discussions were some of the methods employed to determine the usefulness of the CPD knowledge incorporated in student learning activities. Fifty-nine percent of the responses highlighted that the students responded quite positively to the incorporation of CPD activities in their teaching activities.

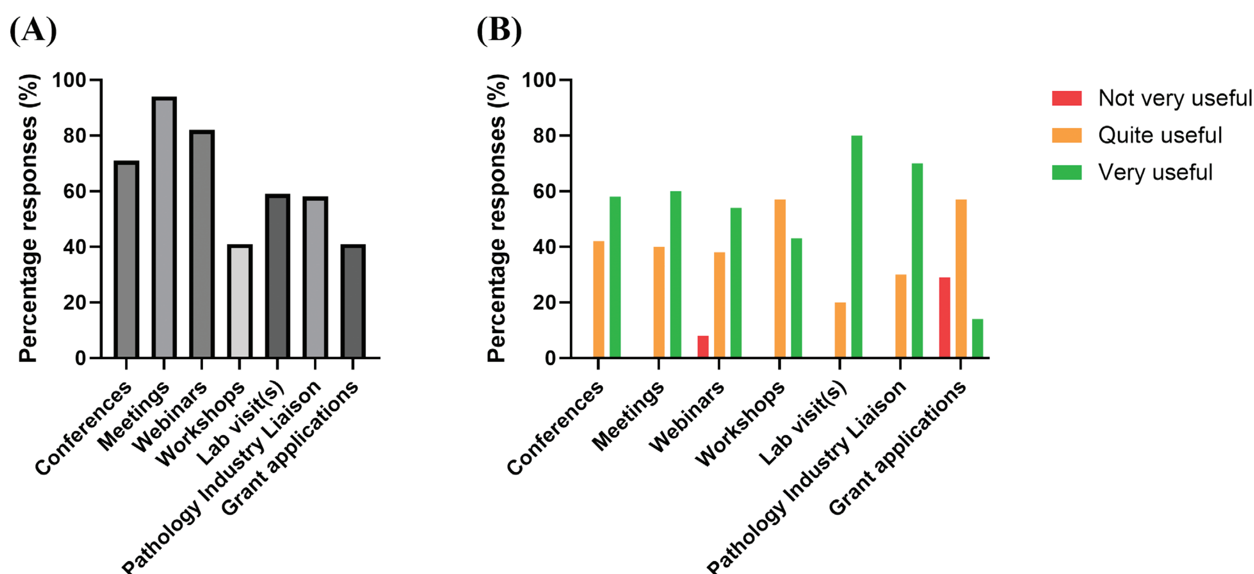

Figure 4. *Key CPD activities engaged by academic staff in the last three years (A) and its usefulness in teaching (B).*

Discussion

The goal of CPD specifically relating to teaching in higher education is to ensure academics maintain currency in professional practice that consequently would be instrumental in delivering work-ready graduates. Studies in the past have been conducted with mixed disciplinary groups of academics and other higher education staff to ascertain various activities undertaken to develop their teaching practices (Ferman 2002; King 2004). For instance, a small-scale research study investigated the experience of academics from a single discipline (Earth Science) across 31 different institutions in the UK (King 2004). Responses from academics indicated that discussions and networking with colleagues were the most frequently cited form of CPD undertaken with several primary barriers identified. The study by King (2004) was also instrumental in highlighting many suggestions and strategies for integrating CPD in discipline-specific teaching. It is therefore crucial to discern the prevailing landscape of academic professional development, as it profoundly influences the delivery of teaching programs. This is particularly important in accredited courses since the currency of teaching practices contributes to the employability and work readiness of graduates.

This study highlights a potential link between academic CPD/currency to professional knowledge and skills of students undertaking AIMS-accredited Medical Laboratory Science programs. Data obtained from phase one and phase two questionnaires were compared to AIMS competency-based standards for medical scientists to propose appropriate recommendations towards CPD opportunities for academic staff. The overall objective was not to emphasize shortcomings in the CPD activities of academics but rather to identify all possible opportunities that could enhance teaching practices that are well aligned to professional needs and AIMS program requirements.

Clinical placement providers identified the capacity to manage extra work demands on supervising staff (91%) and the availability of staff to supervise students (83%) as the main factors contributing to the laboratory's willingness to host students for clinical placements. Comments from surveyed placement providers mentioned staff shortages, being invited to host students, and prioritizing training of new staff over training students on placement as factors contributing to the laboratory's willingness to host students. The majority of placement providers (Figure 1) rated students' understanding as 'limited' on quality control, quality assurance, laboratory standards and guidelines, and laboratory accreditations (NATA, ISO, RCPA), while students' understanding of ethics and workplace health and safety was largely rated 'average'. These results are not unexpected as numerous students would not have previously worked in an accredited medical science laboratory in addition to being on placement for the first time. Most of the students on placement (Figure 2) were observed to have 'good' knowledge of how to follow procedures and protocols, and of personal protective equipment, in addition to applying and adhering to laboratory standards and guidelines. These skills are covered in depth in all undergraduate MLS programs, and it was encouraging to observe students' successful translation of these standard laboratory practices in clinical workplace environments. Students' sampling handling and pipetting skills (Figure 3) were identified as being 'good' however aseptic techniques, microscopy, performing automated and manual tasks in addition to quality control testing were areas that needed improvement. Students' soft skills (teamwork, punctuality, ability to follow directions, communication

skills, responsiveness to critique and feedback) were well developed and thereby students integrated seamlessly into their new workplace environment.

More than 90% of the full-time continuing academics who teach in MLS programs that were surveyed have a graduate degree (Master and/or PhD) in addition to being experienced educators (Table 1). Two thirds (65%) of surveyed academics were AIMS' members (Table 2) and 12% had a current APACE certificate. A third (33%) of surveyed academics did not participate in CPD and academics that participated in CPD attended conferences, meetings, webinars, workshops, and laboratory visits in addition to liaising with the pathology industry and writing grant applications (Figure 4a). The majority of CPD activities undertaken by academics were identified to be very useful (Figure 4b) for teaching purposes and knowledge and/or skills obtained from these CPD activities were incorporated into lectures, course materials, tutorials, practicals, or research.

One-third of surveyed academics acknowledged not participating in CPD and the underlying reasons behind these responses remain unclear. Contributing factors to the neglect of CPD may include academics working long hours in a stressful environment, resulting in sleep deprivation, job dissatisfaction, physical fatigue, and high job pressure (Farahmand *et al* 2022; Nowrouzi-Kia *et al* 2022). Furthermore under their current employment conditions, there often exists no requirement to engage in CPD relevant to their profession. Interactive CPD is known to be more effective than non-interactive CPD and there is no evidence to indicate what the ideal amount of CPD should be despite complex or infrequently used skills can deteriorate between 4-12 months after training (Main and Anderson 2023). CPD should be recognized as a system for supporting and regulating lifelong learning and lifelong education of professionals throughout their working lives (Friedman 2023). In light of this, the Australasian Professional Acknowledgement of Continuing Education (APACE) program is a type of CPD that is highly recommended by AIMS. While academic employment conditions certainly play a significant role in the low uptake of CPD such as the APACE program, it's important to note that APACE is recommended by AIMS as a valuable form of CPD. Despite its endorsement, our findings reveal a notable gap, with only 12% of surveyed academics holding an APACE certificate, despite all being involved in teaching AIMS-accredited courses. This suggests that while APACE may be beneficial, broader systemic factors within academic environments may hinder its widespread adoption. Understanding these dynamics is crucial for enhancing the effectiveness and accessibility of CPD opportunities like APACE within the academic community. Academics without a traditional medical scientist background should be encouraged to complete APACE as this type of CPD will facilitate professional growth that will ensure the currency of MLS skills and knowledge.

Conclusion

The project outcomes have assisted towards obtaining an understanding of the current deficiencies and scope of improvement in MLS teaching delivery. This study highlighted that some academics teaching into accredited MLS programs may not be actively participating in CPD. Based on these findings, it is recommended that all academics teaching within MLS programs actively engage in CPD to ensure they remain current and aligned with professional practices. Overall, the inclusion of formal and informal CPD activities, monitoring and recording of the academic CPD journey that includes planning, reflection and implementation in teaching activities should be packaged through performance review processes for ongoing enhancement of curriculum and student learning.

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Conflicts of Interest

The authors declare they have no conflicts of interest.

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Supplementary 1

Invitation: *You are invited to participate in a research study exploring Continuing Professional Development (CPD) in higher education. The key goal of this project is to understand current CPD of academics involved in teaching inAustralian Institute of Medical and Clinical Scientists (AIMS) accredited Medical Laboratory Science (Pathology) programs in Australia.*

Research Team:

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Charles Sturt University
Email: jramke@csu.edu.au

Why have I been invited to participate?

You have been invited to participate in this study because you have been identified as an industry placement supervisor of students in an AIMS accredited program.

What will I be asked to do?

You are asked to participate in this brief survey to understand the current situation with regards to currency and competency of graduates and current students of accredited medical laboratory science programs. The survey will take about 10-15 minutes. It is being conducted using the Survey Monkey platform (privacy and security policies can be viewed by clicking on these links). Your responses will be collected with others undertaking the survey and then analysed. All data will be anonymous to maintain your privacy.

What are the benefits from participation in this study?

While there are no immediate benefits to participating in this study, understanding the currency and competency of graduates and current students will draw key information that could help assess the current standards of program delivery and identify potential deficiencies (particularly around core clinical departments) that might be linked to currency of academic staff.

Are there any possible risks from participation in this study?

Involvement in this study is considered to be low risk. The survey is not focused on any sensitive or personal topics and, as a result, is not expected to pose any risk or threat to you. Questions and activities are all based around past experiences, are general in nature, and as your participation is voluntary you are free to withdraw at any time. If you are uncomfortable with any question asked, you are free to not respond and/or to seek further clarification as to the purpose of that question.

What if I change my mind during or after studying?

You are free to withdraw at any time and can do so without providing an explanation. If you choose to withdraw from this study, simply close the survey. Any responses that have been entered to that point will have been saved and cannot be removed due to anonymity, however incomplete surveys will be removed prior to data analysis.

What will happen to the information when this study is over?

The data from this study will be completely anonymous in order to maintain your privacy. No personal data is collected or stored as part of this study.

Note: The Charles Sturt University Human Research Ethics Committee has approved this project [Protocol number: H22259].

If you have any complaints or reservations about the ethical conduct of this project you may contact the Committee through the Research Integrity Unit:

Presiding Officer
Human Research Ethics Committee
Charles Sturt University
Locked Bag 588
Wagga Wagga NSW 2650
Ph: (02) 6933 4213
Email: ethics@csu.edu.au

Any issues you raise will be treated in confidence and investigated fully and you will be informed of the outcome. Thank you for considering this invitation.

1. What is your current role?
    - ◯ Laboratory Manager
    - ◯ Laboratory Supervisor
    - ◯ Laboratory Technician
    - ◯ Laboratory Training Officer
    - ◯ Senior Scientist
    - ◯ Pathologist
    - ◯ Other ______________________________

2. Is your laboratory privately or publicly operated?
    - ◯ Public
    - ◯ Private
    - ◯ Other ______________________________

3. Which category/ies best describe the function of your laboratory? (Please select all that apply)
    - ◯ STAT, quick turn-around testing
    - ◯ Multidisciplinary
    - ◯ Single discipline
    - ◯ Other ______________________________

4. When was the last time your laboratory hosted a student on clinical placement?
    - ◯ Less than 1 year ago
    - ◯ 1 - 5 years ago
    - ◯ 6 - 10 years ago
    - ◯ More than 10 years ago

5. On average, how often would your laboratory host a clinical placement?
    - ◯ Each year
    - ◯ Every 2 years
    - ◯ Every 3 - 4 years
    - ◯ Every 5 years or less

6. When hosting a clinical placement, how many students would usually be hosted?
    - ◯ 1
    - ◯ 2 - 3
    - ◯ 4 - 5
    - ◯ More than 5

7. What factors contribute to the laboratory's willingness to host students for clinical placement? (Please select all that apply)
    - ◯ Availability of staff to supervise students
    - ◯ Capacity to manage extra work demands on supervising staff
    - ◯ Students' competency levels
    - ◯ Work availability for students
    - ◯ Other ______________________________

8. What factors contribute to the laboratory's capacity to host students for clinical placement? (Please select all that apply)
    - ◯ Availability of staff to supervise students
    - ◯ Capacity to manage extra work demands on supervising staff
    - ◯ Students' competency levels
    - ◯ Work availability for students
    - ◯ Other ______________________________

9. From your experience with students on clinical placement, how would you rate their understanding of the following concepts? (Please Circle)

Quality control

Very limited / Limited / Average / Good / Very good

Quality assurance

Very limited / Limited / Average / Good / Very good

Workplace health and safety

Very limited / Limited / Average / Good / Very good

Laboratory standards and guidelines

Very limited / Limited / Average / Good / Very good

Laboratory accreditations (NATA, ISO, RCPA, AIM)

Very limited / Limited / Average / Good / Very good

Ethics (e.g. code of conduct, patient confidentiality)

Very limited / Limited / Average / Good / Very good

10. In general, how would you rate the students' knowledge of the following standard laboratory practices? (Please Circle)

Following procedures and protocols
Very limited / Limited / Average / Good / Very good

Personal protective equipment
Very limited / Limited / Average / Good / Very good

Applying and adhering to laboratory standards and guidelines
Very limited / Limited / Average / Good / Very good

11. How would you rate the students' hands-on technical laboratory skills in the following tasks? (Please Circle)

Sample handling (including processing and labelling)
Very limited / Limited / Average / Good / Very good / NA

Pipetting and aliquoting
Very limited / Limited / Average / Good / Very good / NA

Aseptic techniques
Very limited / Limited / Average / Good / Very good / NA

Microscopy
Very limited / Limited / Average / Good / Very good / NA

Performing automated tests (sample loading, working with analysers)
Very limited / Limited / Average / Good / Very good / NA

Performing routine manual tasks (e.g. making blood films, point of care testing, reconstituting analytes, staining, culture and sensitivity testing, blood grouping and antibody ID)
Very limited / Limited / Average / Good / Very good / NA

Performing quality control testing
Very limited / Limited / Average / Good / Very good / NA

12. In your opinion, in what areas of technical laboratory skills could students make improvements?

____

____

13. Please rate how well clinical placement students integrate within your workplace, in the following aspects:

Teamwork
Not at all / Not well / Average / Well / Very well

Punctuality
Not at all / Not well / Average / Well / Very well

Ability to follow directions
Not at all / Not well / Average / Well / Very well

Willingness to comply with directions
Not at all / Not well / Average / Well / Very well

Communication and interpersonal skills
Not at all / Not well / Average / Well / Very well

Responsiveness to critique and feedback
Not at all / Not well / Average / Well / Very well

14. At the end of the student's clinical placement, how often is there potential to offer them employment?

- ◯ Never
- ◯ Sometimes
- ◯ Often
- ◯ Always

15. What type of work would usually be offered?

- ◯ Casual
- ◯ Temporary part-time
- ◯ Temporary full-time
- ◯ Contract
- ◯ Other ______________________________

16. What would be the usual reason/s for not offering employment at the end of a student's clinical placement?

____

17. Please enter any further information, comments or suggestions you would like to add:

____

Supplementary 2

Invitation: *You are invited to participate in a research study exploring Continuing Professional Development (CPD) in higher education. The key goal of this project is to understand current CPD of academics involved in teaching in Australian Institute of Medical and Clinical Scientists (AIMS) accredited Medical Laboratory Science (Pathology) programs in Australia.*

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Why have I been invited to participate?

You have been invited to participate in this study because you have been identified as an educator in an AIMS accredited program.

What will I be asked to do?

You are asked to participate in this brief survey exploring your CPD practice in your current role in an accredited medical laboratory science program. The survey is being conducted using the Survey Monkey platform (privacy and security policies can be viewed by clicking on these links), and will take about 10-15 minutes. Your responses will be collected with others undertaking the survey and then analysed. All data will be anonymous to maintain your privacy.

What are the benefits from participation in this study?

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Are there any possible risks from participation in this study?

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Thank you for considering this invitation.

1. What is the highest level of tertiary qualification you have achieved?
   - ◯ PhD
   - ◯ Masters
   - ◯ Graduate Diploma / Graduate Certificate
   - ◯ Honours
   - ◯ Bachelor degree
   - ◯ Other ______________________________

2. Are you a member of the Australian Institute of Medical and Clinical Scientists (AIMS)?

   ◯ Yes ◯ No

3. What is your current membership level?
   - ◯ Fellow
   - ◯ Full - Professional
   - ◯ Graduate
   - ◯ Member - Non-specific discipline
   - ◯ Member - Discipline specific
   - ◯ Research
   - ◯ Retained
   - ◯ Non-voting - Affiliate
   - ◯ Non-voting - Student

4. Please indicate current memberships you have with any other professional bodies. (Please select all that apply)

◯ AACB	◯ AMS	◯ AVS
◯ RCPA	◯ ICBMB	◯ HGA
◯ ASTH	◯ ASI	◯ HGQ
◯ HSANZ	◯ ICHI	◯ FSA (SIRT)
◯ Blood	◯ ICI	◯ Other
◯ ANZSBT	◯ ISLH	◯ No other current
◯ THANZ	◯ ISTH	
◯ IBMS	◯ ISBT	

5. Do you have a current Australian Professional Acknowledgement Continuing Education (APACE) certificate?

   ◯ Yes ◯ No

6. Have you previously had this certification?
   - ◯ Yes ◯ No

7. When did your most recent certification expire?

   ____

8. What is the status of your current position?
   - ◯ Full time, continuing
   - ◯ Full time, contract
   - ◯ Casual / Sessional
   - ◯ Other ____________________________

9. What is your current academic role?
   - ◯ Level A — Tutor / Associate Lecturer
   - ◯ Level B — Lecturer
   - ◯ Level C — Senior Lecturer
   - ◯ Level D — Associate Professor
   - ◯ Level E — Professor
   - ◯ Other ____________________________

10. Is this role part of a professionally accredited (AIMS) program / course?
    - ◯ Yes ◯ No

11. How long have you been teaching in this program / course?
    - ◯ 1 year or less
    - ◯ 2 - 5 years
    - ◯ 6 - 10 years
    - ◯ 11 - 20 years
    - ◯ More than 20 years

12. Over your career, how long have you taught in professionally accredited (AIMS)
    - ◯ programs / courses?
    - ◯ Not at all
    - ◯ 1 - 5 years
    - ◯ 6 - 10 years
    - ◯ 11 - 20 years
    - ◯ More than 20 years

13. Are you involved in peer reviewing journal manuscripts?
    - ◯ Yes ◯ No

14. On average, how often do you peer review journal manuscripts?
    - ◯ 1 or 2 per year
    - ◯ 3 - 5 per year
    - ◯ More than 5 per year

15. Do you participate in continuing professional development (CPD) activities related to Medical Laboratory Science / Pathology?
    - ◯ Yes ◯ No

16. On average, how many CPD activities do you enrol in and/or attend annually?
    - ◯ 1 ◯ 2 - 3 ◯ 4 - 5 ◯ More than 5

17. Please indicate all the CPD activities you have attended or participated in over the last three years:
    - ◯ Conferences ◯ Meetings
    - ◯ Webinars ◯ Workshops
    - ◯ Lab visit(s) ◯ Pathology Industry Liaison
    - ◯ Grant applications

18. Of the Professional Development activities you have attended, at what level were they? (Please circle)

Conferences
    Special Interest Group / State / National / International

Meetings
    Special Interest Group / State / National / International

Webinars
    Special Interest Group / State / National / International

Workshops
    Special Interest Group / State / National / International

Lab visit(s)
    Special Interest Group / State / National / International

Pathology Industry
    Special Interest Group / State / National / International

Liaison
    Special Interest Group / State / National / International

Grant applications
    Special Interest Group / State / National / International

19. Other than the CPD activities previously mentioned, please list any others you participate in, such as scientific groups, clubs, societies, etc.

    ____

    ____

20. How useful for your teaching have you found each of the CPD activities you have attended or participated in? (Please circle)

Conferences
    Not very useful / Quite useful / Very useful

Meetings
    Not very useful / Quite useful / Very useful

Webinars
    Not very useful / Quite useful / Very useful

Workshops
    Not very useful / Quite useful / Very useful

Lab visit(s)
    Not very useful / Quite useful / Very useful

Pathology Industry Liaison

Not very useful / Quite useful / Very useful

Grant applications

Not very useful / Quite useful / Very useful

21. How were the knowledge or skills gained from these CPD activities utilised? Please indicate any of the following into which they were incorporated:

◯ Lectures
◯ Course notes or materials
◯ Tutorials
◯ Practicals
◯ Other ______________________________

22. How helpful did you find the incorporation of your CPD activities in the following areas:

Useful tools for teaching

Very limited use / Slightly useful / Moderately useful
Very useful / Extremely useful

Added value to teaching

Very limited use / Slightly useful / Moderately useful
Very useful / Extremely useful

Improved quality of teaching

Very limited use / Slightly useful / Moderately useful
Very useful / Extremely useful

Enhancing students' capacity for learning

Very limited use / Slightly useful / Moderately useful
Very useful / Extremely useful

Improving students' overall learning and assessment performance

Very limited use / Slightly useful / Moderately useful
Very useful / Extremely useful

23. What methods did you use to determine the usefulness of the CPD activities you incorporated into your teaching? (Please select all that apply)

◯ Student assessment performance review
◯ Quiz exercise/s
◯ Case studies
◯ Group discussion/s
◯ Other ______________________________
◯ No review was done

24. Please rate how well students responded to the incorporation of your CPD activities?

◯ Not well No difference
◯ Slightly positive
◯ response
◯ Quite positive
◯ response
◯ Very positive
◯ response

25. How did you determine students' responses to the incorporation of of the CPD activities into your teaching materials? (Please select all that apply)

◯ Subject review survey (students' comments and feedback)
◯ Noticeable improvement in students' overall understanding of subject content and lecture materials
◯ Noticeable improvement in students' participation and performance in tutorials
◯ Noticeable improvement in students' practical understanding and performance
◯ Noticeable improvement in students' assessment performance
◯ Overall academic grade improvement
◯ Other ______________________________
◯ No difference was noticed.

26. Please enter any further information, comments or suggestions you would like to add:

____

TECHNICAL NOTE

Preferred terminology for the description of cytotoxic T cells: good professional practice for the Australian Standard AS ISO 15189:2023 accredited medical laboratory

Dennis Mok[1], Naira Eloyan[2], Rana Nabulsi[3], Sharfuddin Chowdhury[4], Georgi Koutin[5], Amanda Neill[6]

[1]Medical Management Consulting, Birkdale, Queensland, Australia

[2]Scientific Center of Drug and Medical Technology Expertise, Yerevan, Armenia

[3]Dubai Academic Health Corporation, Dubai, United Arab Emirates

[4]King Saud Medical City, Riyadh, Saudi Arabia

[5]Role 2B Medical Treatment Facility, Camp Film City, Pristina, Kosovo

[6]Aspen Pharmacare Australia, St. Leonards, NSW, Australia

The contemporary issue of describing cytotoxic T cells

In medical laboratories accredited to the Australian Standard AS ISO 15189:2023, all examination reports must include relevant information that is accurate, clear and unambiguous for the interpretation of examination results. This supports harmonisation and standardisation that has the obvious benefits of improving the interpretation of pathology reports leading to minimisation of errors in diagnoses and treatments. The medical laboratory must ensure all of the terms for reporting are in alignment with current good practice by establishing internal guidelines to determine what is practical for laboratory users. This also applies to haematological light microscopic analysis of lymphocytes (Figure 1). Currently there are three terms that are used to refer to cytotoxic T cells. This technical note aims to provide a justified recommendation for the AS ISO 15189:2023 accredited medical laboratory.

Declared descriptions from international and national organisations

Cytotoxic T cells are currently termed differently by various organisations. The terms include 'variant lymphocyte', 'atypical lymphocyte', and 'reactive lymphocyte'. The Area Committee on Hematology, Subcommittee on Qualitative Cellular Hematology at the Clinical and Laboratory Standards Institute (CLSI) in the United States recommends the use of variant lymphocyte rather than atypical or reactive lymphocyte, so the CLSI preferred term is variant lymphocyte (CLSI 2007). The CLSI is an internationally oriented national organisation. The term variant lymphocyte was adopted by the Regenstrief Institute, Logical Observation Identifiers Names and Codes (LOINC) Committee to develop terminologies for medical laboratories and observations and therefore is the preferred term for that organisation. The LOINC has become a language by using universal codes, unique identifiers, and structured names for identifying health measurements, observations, and document (Baorto *et al* 2022). The Royal College of Pathologists of Australasia (RCPA) established the Standards for Pathology Informatics in Australia (SPIA) to improve the standard of pathology requesting and reporting in Australia and New Zealand to reduce errors from terminological usage variations (RCPA 2024). The RCPA released a practice guideline that included SPIA preferred terms for all pathology requests and reports to support the National Pathology Terminology and Information Standardisation Plan (RCPA 2021). The RCPA SPIA preferred term is atypical lymphocyte, rather than the LOINC preferred term. The term atypical lymphocyte is listed in the RCPA SPIA Haematology and Transfusion Medicine Reference Set (Version 4.0) and is distributed by the National Clinical Terminology Service, operated by the Australian Digital Health Agency. Finally, the International Council for Standardization in Haematology (ICSH), Committee on Standardization of Peripheral Blood Cell Morphology, published recommendations for the standardisation of nomenclature and grading of peripheral blood cell morphological features. The ICSH preferred term is reactive lymphocyte (Palmer *et al* 2015). In summary, there are three options available for the description of cytotoxic T cells.

Address correspondence to:
Sharfuddin Chowdhury
Email: s.chowdhury@ksmc.med.sa

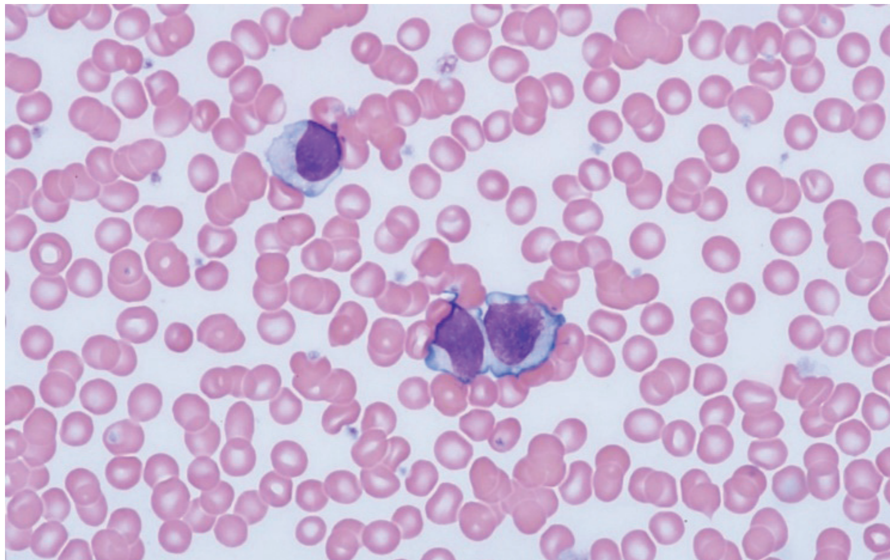

***Figure 1.** Light microscopic analysis of reactive lymphocytes. Three large reactive lymphocytes with deeply basophilic cytoplasm in a case of infectious mononucleosis. Wright stain at pH 6.8, magnification 1000x.*

Analysis of justified course of actions

Course of action 1

The medical laboratory adopts the preferred terms from the RCPA SPIA Haematology and Transfusion Medicine Reference Set and thus uses the term 'atypical lymphocyte'. Course of action 1 is in alignment with the RCPA recommendation that examination reports should use preferred terms from the RCPA SPIA Pathology Terminology Reference Sets (RCPA 2024). This course of action is in partial alignment with the medical laboratory's commitment to good professional practice, as specified in Clause 5.5 a) of AS ISO 15189:2023; however, the implementation of course of action 1 fulfils the RCPA recommendations.

Course of action 2

The medical laboratory adopts the ICSH preferred terms and thus uses the term 'reactive lymphocyte'. This course of action is not in alignment with the current RCPA recommendation that examination reports should use preferred terms from the RCPA SPIA Haematology and Transfusion Medicine Reference Set; however, this course of action is in alignment with the International Laboratory Accreditation Cooperation mutual recognition arrangement to which the National Association of Testing Authorities, Australia is a current signatory. Examination reports should use internationally accepted haematology terms. The implementation of course of action 2 fulfils the ICSH recommendations; therefore, examination reports referring to reactive lymphocytes are internationally identifiable and in full alignment with the medical laboratory's commitment to good professional practice, as specified in Clause 5.5 a) of AS ISO 15189:2023.

Acknowledgements

The authors would like to thank: Gillian Rozenberg, FAIMS FFSc (RCPA), Principal Scientist, South Eastern Area Laboratory Services, Prince of Wales Hospital, Randwick, Australia, for providing the micrograph; Tina Pham, BAppSc FAIMS, Senior Scientist in Special Haematology, St. Vincent's Hospital, Fitzroy, Australia; and Helen Neilson, BSc (Hons) CQP MCQI RCT, Quality Manager, NHS Greater Glasgow and Clyde, Glasgow, United Kingdom, for reviewing the manuscript.

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Clinical and Laboratory Standards Institute 2007. *Reference leukocyte (WBC) differential count (proportional) and evaluation of instrumental methods; approved standard—Second edition.* 2nd ed. Wayne: Clinical and Laboratory Standards Institute

Palmer L, Briggs C, Mcfadden S, Zini G, Burthem J, Rozenberg G, Proytcheva M, Machin SJ 2015. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. *Int J Lab Hematol* 37: 287-303.

Royal College of Pathologists of Australasia 2021. *Best practice guideline.* Surry Hills: Royal College of Pathologists of Australasia.

Royal College of Pathologists of Australasia 2024. *Standardised pathology informatics in Australia (SPIA) guidelines.* Version 4.1. Surry Hills: Royal College of Pathologists of Australasia.

The AIMS APACE CPD scheme is a voluntary program that recognises continuing education, formal courses, and a wide range of professional activities which contribute to professional growth.

- Meet your ISO 15189 CPD requirements.
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A P A C E

Australian Professional Acknowledgement of Continuing Education (APACE)

3 APACE credits per set of questions will be awarded if at least 8 out of 10 questions are answered correctly.

Journal-based CPD No. 100
Page 1 of 2

Questions relating to the article *'β-lactam resistance in* Pseudomonas aeruginosa *and its detection in the microbiology laboratory'* at page 126 of this issue.

1	β-lactam antibiotics are commonly the first line treatment option for P. aeruginosa infections.	True/False
2	*Pseudomonas aeruginosa* is a Gram-positive rod.	True/False
3	P. aeruginosa is capable of harbouring a wide range of antimicrobial resistance mechanisms, often simultaneously, leading to strains that are resistant to multiple antibiotic classes.	True/False
4	Carbapenem resistance is not considered a major global public healthcare issue.	True/False
5	The mechanism of action of β-lactams antibiotics is to interrupt bacterial cell-wall formation.	True/False
6	Phenotypic testing provides information on which antimicrobial will either kill or arrest growth of an organism.	True/False
7	Genotypic AST attempts to identify specific resistance genes or genetic mutations using molecular or genomic methods.	True/False
8	AGAR, NAUSP, NPAS and APAS contribute to AURA.	True/False
9	β-lactam resistance is caused by transmissible mechanisms, and are therefore unavoidable.	True/False
10	Antimicrobial susceptibility testing (AST) is performed to assess the ability of an antimicrobial agent to inhibit or kill an organism *in vivo.*	True/False

Name: ____

Email: ____

Please photocopy this page or print it from the electronic AJMS which is stored in the AIMS 'Member Centre' under the heading 'Journal' at www.aims.org.au. ***Circle your answers, then scan, and enter them as activities in the APACE diary under 'My CPD Record'.***

https://www.aims.org.au/online-diary

A P A C E

Australian Professional Acknowledgement of Continuing Education (APACE)

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Journal-based CPD No. 101
Page 2 of 2

Questions relating to the article *'Comparison of laboratory coagulation tests and thromboelastography and their influence on transfusion algorithms in cardiac surgery'* at page 146 of this issue.

1	Cardiac surgeries, particularly those requiring the use of cardiopulmonary bypass (CBP), often result in perioperative bleeding requiring transfusion of blood products.	True/False
2	The TEG measures clotting on a whole blood sample collected in a citrate tube.	True/False
3	According to ANZBT guidelines, a massive transfusion is defined as either transfusion (in an adult person) of more than one blood volume (i.e. 10 units in 24h) or in acute situations transfusion of half the blood volume (five units) in 4 h.	True/False
4	TEG uses the principle of viscoelasticity to measure coagulation initiation, clot formation, clot strength and fibrinolysis.	True/False
5	In traditional laboratory coagulation the PT/INR and APTT are the most commonly performed coagulation screening tests.	True/False
6	In general, the TATs for TEG are slower than traditional laboratory tests.	True/False
7	In this study, the weakest correlations with the normal TEG tests were between CK-R time and INR.	True/False
8	TEG algorithms in cardiac surgery show a reduction in blood product usage and better outcomes for patients.	True/False
9	All sites use the same transfusion algorithms and the same TEG parameters and thresholds to guide transfusion.	True/False
10	The TEG® 6s is a 4 channel POC analyser that includes Normal TEG, Rapid TEG, Heparinase TEG and Functional Fibrinogen TEG.	True/False

Name: ____

Email: ____

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Australasian
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Education

Recognition of
Continuing Professional Development

Australian Institute of
Medical and Clinical Scientists

www.aims.org.au **www.apace.org.au**

The APACE (Australasian Professional Acknowledgement of Continuing Education) scheme is a voluntary programme that recognises continuing education, formal courses and a wide range of professional activities which contribute to your professional growth.

The healthcare industry is undergoing rapid change. We are expected to keep our knowledge and skills up to date to enable us to perform to the highest professional standard. The APACE scheme provides a method by which your professional activities are recognised.

Changes to APACE due to COVID-19 pandemic UPDATE

An APACE Certificate is usually awarded on attaining 100 CEU credits within a two year period.

As webinars and online conferences, meetings and workshops are all interactive, it was considered that this is the same as attending in person, therefore the same number of points will be awarded for attendance either virtually or face-to-face. This should enable more members to attend as no travelling time, costs and in some cases the online attendance with be without cost to the attendee.

Therefore, the extended time frame due to Covid-19 will no longer apply.

https://www.aims.org.au/apace

Australian Institute of Medical Scientists

Immunohaematology Quality Assurance Program

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- Designed to suit both small and large laboratories;
- Includes blood grouping, antibody screening/identification and compatibility testing;
- Runs bi-monthly starting in July at the beginning of each financial year;
- The reports provide participants' own results along with graphical representation of results of their peers (de-identified) allowing for easy comparison and analysis.

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Instructions to authors

The following instructions are based on the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals", also known as the Declaration of Vancouver, and on the *Australian Government Style manual: for authors, editors and printers*, 6th edition, 2002. URLs were correct on September 29th, 2008.

Manuscripts that do not fully comply with the following 'Instructions to Authors' may be returned for revision before they are considered for publication.

The *Australian Journal of Medical Science (AJMS)* will consider for publication any paper relevant to the field of Medical Science. Disciplines include blood banking, clinical biochemistry, haematology, histopathology, immunology, microbiology and molecular biology. Areas of general interest to medical laboratory scientists, including toxicology, epidemiology, public and community health, and professional and management issues will also be considered.

Papers published in the *AJMS* are in the form of:

- Review Articles
- Original Articles
- Brief Communications
- Technical Notes
- Case Studies
- Letters to the Editor
- Book Reviews

Articles submitted for publication are understood to be offered only to the *AJMS* and those accepted become the property of the *AJMS*.

All individuals listed as authors must have made a substantial contribution to the conception and design of the study, the acquisition of data or the analysis and interpretation of data; the drafting of the article or revising it critically for important intellectual content; and final approval of the version to be published. The corresponding author must take responsibility for obtaining permission from all the authors for the submission of any version of the manuscript and for any changes in authorship.

When the manuscript is submitted the authors must disclose any potential conflict of interest and/or commercial support.

Requirements & preparation of manuscripts

General

Articles should be submitted in electronic format to programs@aims.org.au. If an article is too large to be submitted by email, it should be submitted on an or USB stick.

Number pages consecutively commencing with the title page.

Arrange the article in the following sequence:

- Title page
- Abstract and key words
- Main Text
- Acknowledgements
- References
- Tables - each table, complete with title and footnotes, on a separate page
- Legends for illustrations.

Authors should ensure that their manuscript communicates their ideas and concepts simply and clearly so that the article is easily read and understood. Authors are strongly recommended to refer to the recommendations on reporting standards as outlined in the statements and checklists of the CONSORT group (see: http://www.consort-statement.org/) and similar groups such as STARD (see: http://www.stard-statement.org/). The principles outlined in these standards may be used as general guidelines and not just as applied to clinical trials and diagnostic studies.

Title page

The title of the article should not exceed three lines (40 characters per line), including punctuation and spacing. All authors must be identified on the title page (e.g. William Smith, Susan Yeo, …"). Where applicable, the title page should also include the name of the institution with which each author is affiliated and to which the work should be attributed. In the case of multiple authors, the name, postal address, email address, telephone and facsimile number of the author responsible for correspondence relating to the manuscript should be indicated.

Abstract & keywords

The abstract should be approximately 150 words and should make sense when read alone or in conjunction with the article. The abstract should be a concise overview that describes the important details of the article including the purpose of the study/investigation, basic procedures (study subjects/experimental animals/observational and analytic methods) and the results and principal conclusions. New and important aspects of the work and its implications may also be included. References should not be included.

Three to ten keywords may be listed. Authors are advised to comply with the terms from the Medical Subject Headings (MeSH) list from Index Medicus (see http://www.nlm.nih.gov/mesh/). Keywords should be given below the Abstract.

Text

The style of writing should conform to acceptable English usage. Do not use slang, medical jargon or unnecessary abbreviations. Accepted spelling is the first choice given in the latest edition of the Macquarie Dictionary.

Wherever possible, observational or experimental articles should be divided into sections headed:

- Introduction
- Materials and methods
- Results
- Discussion
- References

For other types of articles such as commentaries, reports and reviews, use an appropriate format or consult the Editors for guidance. Do not include a separate section for conclusions, these should be given in the discussion.

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Clearly state the purpose of the article leading the reader from the known to the unknown. Summarise the rationale for the study and state the question to be answered as appropriate. Give only strictly pertinent references, and do not review the subject extensively.

Materials & methods

Present the materials and methods in a logical sequence. Describe the selection of the observational or experimental subjects (patients or experimental animals, including controls) clearly. Notification of ethics approval must be given where relevant. Identify the methods, apparatus and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods. Adequately describe new or substantially modified methods. Identify precisely all drugs and chemicals used, including generic name(s), dosage(s), and route(s) of administration. Do not identify patients or hospitals without consent.

Results

Present the results in the same sequence as given in the Materials and methods; use tables and illustrations where these will help the reader understand the work being presented. Do not repeat in the text all the data in the tables or illustrations.

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Indicate the new and important aspects of the study and emphasise the conclusions that follow. Do not repeat in detail data given in the Results section and do not add new data. Include in the Discussion the implications of the findings and their limitations and compare the observations to other relevant studies. Recommendations may be included if appropriate. Link the conclusions with the goals of the study and answer the experimental question stated in the Introduction. However, avoid unqualified statements and conclusions not completely supported by your data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly label them as such.

Acknowledgements

Acknowledge individuals who have made substantial contributions to the study including technical work and financial support. Authors are responsible for obtaining consent from all the individuals acknowledged by name as inclusion may be interpreted as an endorsement of the article's contents.

References

The AJMS uses a modified Harvard System (author-date system).

Throughout the body of the manuscript cite the author/s name and the publication year in parentheses as in the following examples:

(i) Research in this area (Jones 1999) …

(ii) It has been successfully demonstrated that (Smith and Brown 1981; Auteur 1995; Scienziato *et al* 2007).

(iii) Following further investigation, Wetenschapper (2002 highlighted the difficulties inherent in…

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Examples of the correct form for references are given below:

Journal Reference:

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Personal Author(s) of a book:

Osler AG 1976. *Complement: mechanisms and functions*. Englewood Cliffs: Prentice-Hall.

Editor, Compiler, Chairman as Author:

Rhodes AJ, Van Rooyen CE, comps. 1968. *Textbook of virology: for students and practitioners of medicine and the other health sciences*. 5th ed. Baltimore: Williams and Wilkins.

Chapter in Book:

Weinstein L, Swartz MM 1974. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, eds. *Pathologic physiology: mechanisms of disease*. Philadelphia: WB Saunders; 457-472.

Online documents:

National Center for Biotechnology Information. OMIM: online Mendelian inheritance in man. http://www.ncbi.nlm.nih.gov/omim. Accessed February 25, 2007.

Tables

Number tables consecutively with Arabic numerals and supply a brief title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in headings. Explain in footnotes all non-standard abbreviations used in each table.

For footnotes, use the following symbols in this sequence:

* † ‡ § ¶ ** ††

In preparing tables, consideration should be given to the page width of the Australian Journal of Medical Science. All tables should be prepared for publication vertically. In the text, cite each table in consecutive order, and mark in the margin of the text its approximate location.

If data from another published or unpublished source is used, written permission must be obtained and a copy must accompany the manuscript.

Illustrations

Colour illustrations may be submitted on a USB stick. Images should be scanned at a minimum of 300 dpi.

When plotting points, the following symbols are preferred:

In most instances, figures will be reduced to one column in width. All letters and numbers should be drawn to be at least 1.5 mm high after reduction, symbols at least 1.0 mm. Titles for illustrations belong in the legends for illustrations and not on the illustrations themselves.

Photomicrographs must have internal scale markers and the magnification must be stated. Symbols, arrows, or letters used in the photomicrographs should contrast with the background.

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When symbols, arrows, numbers, or letters are used to identify parts of illustrations, identify and explain each one in the legends. The figure legend must contain a boldface (a) name ("Figure" + arabic figure number) and (b) substantive title.

Abbreviations

Use only standard abbreviations (see list of commonly used abbreviations).

Avoid abbreviations in the title. The full term for which an abbreviation stands must precede its first use in the text unless it is a standard abbreviation for a unit of measurement.

Report measurements in the units in which the measurements were made. In most countries the International System of Units (SI) is standard.

Commonly used abbreviations

Abbreviation or Symbol	Standard Units of Measurement
g	gram
g	gravity
Hz	hertz
h	hour
IU	international unit
K	kelvin
kg	kilogram
L	liter, litre
m	meter, metre
min	min
M	molar
mL	millilitre
mol	mole
N	newton
nm	nanometre
p	probability
rpm	revolutions per min
s	second
wk	week
yr	year

Additional information

The following are useful sources of information. The first two publications are used by the AJMS as standard references.

Style Manual Committee. Council of Biology Editors. *Scientific style and format: the CBE manual for authors, editors, and publishers.* 6th ed. Cambridge University Press, 1994.

Style manual for authors, editors and printers. 6th ed. John Wiley & Sons Australia Ltd, 2002.

O'Connor M, Woodford FP. *Writing scientific papers in English: an ELSE-Ciba Foundation guide for authors.* Amsterdam, Oxford, New York: Elsevier-Excerpta Medica, 1975.

Day RA. *How to write and publish a scientific paper.* Philadelphia, Institute for Scientific Information Press, 1979.

Zeiger M. *Essentials of writing biomedical research papers*. 2nd ed. New York, McGraw-Hill, 2000.

Matthews JR, Matthews RW. *Successful scientific writing: a step-by-step guide for the biological and medical sciences*. 3rd ed. Cambridge, Cambridge University Press, 2007 [Also available in eBook format.]

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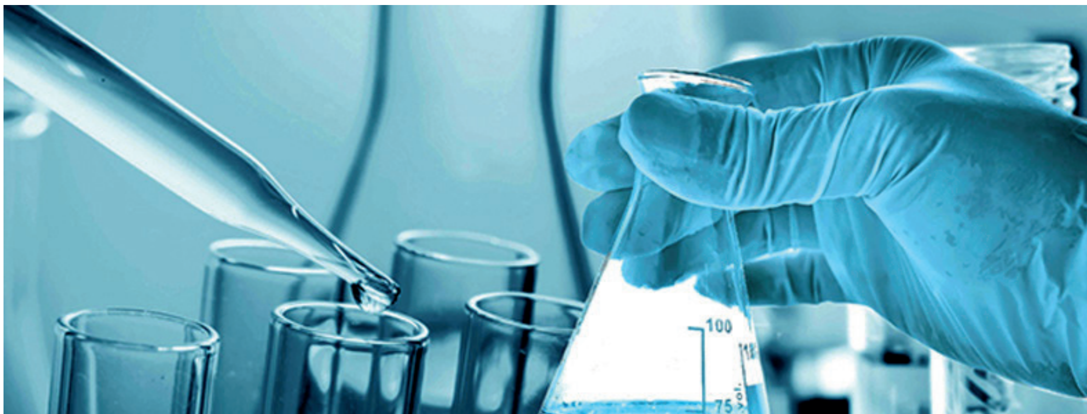

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Changes to Certification arrangements for the Medical Laboratory Science Profession

From April 2023, the Australian Council for the Certification of the Medical Laboratory Scientific Workforce (CMLS) Board are no longer accepting applications for certifications directly. Instead, professional bodies operating CMLS approved CPD schemes will be able to issue certification on behalf of the Council for their members who meet the requirements for certification as detailed on the CMLS website.

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